

PROGRESS REPORT

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Project Title: Integration of neurobiological and computational analyses of the neural network essentials for conditioned taste aversions

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Principal Investigator: Dr. Kathleen C. Chambers

GOALS

The general goal of the ONR project is to determine the neural basis of learning and memory, i.e., how the brain stores and retrieves memory. More specifically we are determining how the hard-wired (innate) part of the neural system interfaces with the plastic (learned) part. The special form of learning which is the focus of this project is conditioned taste aversions (CTAs), i.e., learned aversions to the taste of a food or fluid when consumption of that substance is followed by illness. In order to achieve this general goal, neurobiological and computational analyses of the neural network essentials for CTA are being integrated. The essential neurobiological network for CTA is being identified and characterized and computational models for the CTA neural circuit are being developed. JZ

NEUROBIOLOGICAL RESEARCH

It is necessary to identify four pathways in order to gain a clear understanding of the neural basis of CTAs: the US (illness) pathway, the CS (taste) pathway, the pathway for the elicited response to the CS prior to conditioning (UR_{CS} or unconditioned ingestive response, UIR), and the pathway for the elicited response to the CS after conditioning (CR_{CS} or unconditioned aversive response, UAR). As much work has already been done on the taste and UR_{CS} pathways, we are concentrating on the illness and illness-taste integration pathways in this proposal. In addition, we also are identifying endogenous factors that modulate the acquisition and extinction of conditioned taste aversions.

Illness Pathway

There are two known detection systems for toxins, the gastric-intestinal mucosa and the area postrema. The vagus nerve conveys information from the gastric-intestinal mucosa to the nucleus of the solitary tract (NST), pontine parabrachial nucleus

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(PBN) and the insular cortex (Cechetto & Saper, 1987; Norgren, 1978; Torvik, 1956). The area postrema detects chemicals in the blood and is thought to convey this information to the NST (Morest, 1967). Beyond this little is known about the illness pathways.

A wide variety of substances can induce CTAs. The detection system that is used to convey information about these substances to the brain varies with the particular chemical and the route of administration. LiCl, a widely used illness-inducing agent, acts by way of the area postrema; copper sulfate acts by way of the vagus nerve when it is administered intraperitoneally; and, apomorphine acts by systems other than the area postrema and the vagus nerve.

One of our aims is to determine the conditions under which endogenous substances act as illness-inducing agents in a CTA and to determine the neural pathways by which these agents and the commonly found toxin, LiCl, produce their effect.

Experimental Series 1: Nature of Toxin

We have completed 3 experiments aimed at determining the role of estradiol in conditioned taste aversions and its possible toxic effects. The manuscript that was submitted to Physiology and Behavior was accepted for publication (see Appendix A) and new data were presented at the American Psychological Society meetings in June. In addition, one other experiment has been completed and one is underway.

We have demonstrated that estradiol prevents testosterone from prolonging extinction of a conditioned taste aversion when it is administered during acquisition or during extinction (Appendix A). Since testosterone is effective in slowing extinction only when it is present during extinction, estradiol does not have to be present concurrently with testosterone during extinction to be effective. This suggests that estradiol does not act on a testosterone-related mechanism but rather acts independently of testosterone.

If estradiol acts independently of testosterone, then one would expect that estradiol would increase extinction rate regardless of the presence of testosterone during extinction. We have found that when only estradiol is given during acquisition and extinction of a conditioned taste aversion, extinction is accelerated (see Appendices B and C).

If the action of estradiol alone is the same as when it is given with testosterone, then it should accelerate extinction when administered during acquisition and during extinction. A study is presently underway to test this.

Experimental Series 2: Neural Pathways

We decided to concentrate our efforts on the role of the



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area postrema in mediating the effects of estradiol and other toxins. Thus we temporarily put aside pursuing the 2-DG studies to identify active areas of the brain after administration of illness-inducing agents and the multiunit recording from the PBN and gustatory cortex. We have refined our techniques for lesioning the area postrema. This summer and fall we will conduct a study to determine whether the area postrema is essential for observation of the estradiol effects. We also will begin to learn a temporary lesioning technique whereby the area postrema will be cooled during estradiol administration.

Estradiol is known to produce nausea and vomiting in humans. Recently, it has been reported in other labs that increases in estradiol can induce the development of learned food aversions to available foods (Bernstein & Fenner, 1983). This effect is attenuated after lesions of the area postrema (Bernstein et al., 1985, 1986). We have found that estradiol accelerates extinction of an aversion and prevents testosterone from prolonging extinction. It is possible that estradiol produces these effects because of its toxicity. If this is the case, then lesions of the area postrema should attenuate the effects of estradiol on extinction of conditioned taste aversions. Experiments will be conducted to determine the role of the area postrema in estradiol modulation of conditioned taste aversions.

Illness-Taste Integration Pathway

We have completed 4 experiments aimed at identifying neural areas mediating illness-taste integration.

Experimental Series 1: Role of the Amygdala

Several brain structures have been implicated in CTAs, but until recently, lesions of the amygdala (AMG), in particular the basolateral AMG, have produced the most consistent findings; they disrupt acquisition and retention of prelesion CTAs (Aggleton et al., 1981; Nachman & Ashe, 1974; Simbayi et al., 1986). But after finding that cutting the connections between the AMY and the temporal cortex produced the same deficits as lesions of the basolateral AMG, Fitzgerald and Burton (1983) suggested that it is the destruction of the fibers of passage that produces the deficits after lesions of the basolateral AMG and not the destruction of the nucleus itself. Recently, Dunn and Everitt (1988) found that neurotoxic (ibotenic)-induced lesions which spare the fibers of passage had no effect on aversion learning whereas electrolytic (ELEC) lesions which destroy both cells and fibers attenuated the aversion. Neither ibotenic or ELEC lesions had a significant effect on extinction. They concluded that it is the axons passing between the brain stem/hypothalamus and GN that are responsible for the deficits in acquisition after ELEC lesions of the AMG. In the following study, we set out to examine the effect of ELEC and neurotoxic (NMDA, N-methyl-D,L-aspartic acid)-induced lesions on acquisition and extinction of a CTA.

We have completed all behavioral testing and histological examination for two experiments (see Appendix D). Acquisition was attenuated in rats with ELEC lesions but not NMDA lesions when lesions were made before acquisition or after acquisition. Neither ELEC or NMDA lesions affected extinction. These results suggest that the basolateral AMG does not play a role in acquisition or extinction of conditioned taste aversions.

Experimental Series 2: Role of the Gustatory Cortex

Animals with lesions of the gustatory cortex (GN) exhibit slower acquisition of CTAs (Braun et al., 1972) and no retention of a prelesion CTA (Braun et al., 1981; Kiefer et al., 1984; Yamamoto et al., 1980). The effect of GN lesions on extinction is unclear. We have completed two studies designed to determine the effect of GN lesions on acquisition of a CTA. In the first experiment the lesion was made before acquisition of a CTA and in the second experiment lesions were made after acquisition. In both experiments male rats were randomly divided into 2 groups: sham control and lesion (by aspiration). The results from both experiments indicate that there is no effect of GN lesions on acquisition of a CTA. As these results differ from previous reports, an analysis of differences between those studies and ours will be made. Some obvious differences include the extent of the lesion, strain of rat, and fluid deprivation state.

Modulating Factors

We have completed 7 experiments aimed at identifying factors that modulate CTAs. Two of these experiments have been combined in a paper that has been accepted for publication in Behavioral Neuroscience (Appendix E). One of the experiments was presented at the Western Psychological Association meeting in April (Appendix F), and one was presented at the American Psychological Society meeting in June (Appendix C). Two other experiments have been combined and part of the results have been submitted for presentation at the American Psychological Association meeting and the other part for presentation at the Gerontological meeting (Appendices G and H).

Experimental Series 1: Hormonal Effects on Conditioned Taste Aversions

Effects of perinatal testosterone on conditioned taste aversion extinction. We have found that the rate of extinction of a CTA in rats is dependent on concurrent levels of testosterone. When testosterone is administered to gonadectomized males and females, extinction is prolonged. However, females are less sensitive to testosterone than males. We have found that it is the presence of testosterone during the perinatal period that alters sensitivity to testosterone. Adult gonadectomized rats that had low levels of testosterone present during the perinatal period (normal females) exhibited a fast rate of extinction when given a low dose of testosterone whereas adult gonadectomized rats that had testosterone present during the

perinatal period (males and androgenized females) showed a slow extinction rate when given the same low dose. Thus, although the presence of testosterone during the perinatal period is not critical for the expression of a slow extinction rate, it does reduce the amount of testosterone required to produce the slow rate (Appendix E, accepted for publication).

Effects of gonadectomy on extinction of a conditioned food aversion. Gonadal hormones alter the rates of extinction of conditioned food aversions in rats. Males have slower extinction rates than females. Gonadectomy increases the rates in males but has no effect in females. Testosterone treatment slows extinction in both males and females. We have observed that gonadectomy increases the extinction rates of males to those of females in Sprague-Dawley but not Fischer 344 rats. In studies of reproductive behavior, the decrease in sexual activity after gonadectomy occurs over a long period of time. One possible explanation for the difference in the effects of gonadectomy in Sprague-Dawley and Fischer 344 rats is that gonadectomy may take longer to show an effect in Fischer 344. The following experiment was designed to determine whether the extinction rates of Fischer 344 rats varies with the length of time after gonadectomy. A conditioned food aversion was induced in 20 males and 20 females one week or 5 weeks after gonadectomy. Following the first presentation of a 10% sucrose solution, the conditioned food aversion was induced by injection of LiCl (0.15 M, 10 ml/kg). Daily extinction trials began 2 days later and continued until criterion for extinction (100% of first day consumption) was reached. The extinction rates of the 5-week males were significantly faster than those of the 1-week males ($p < 0.05$). The extinction rates of the two groups of females did not differ. Both groups of males, however, still exhibited slower extinction rates than both groups of females ($p < 0.05$). These results suggest that differences in the effects of gonadectomy on extinction rates in Sprague-Dawley and Fischer 344 rats may be accounted for, at least in part, by differences in the length of time the effects of gonadectomy are expressed (Appendix F, presented at the Western Psychological Association meeting, April, 1990).

Experimental Series 2: Age-Related Effects on Conditioned Taste Aversions

Age-related changes in sensitivity to estradiol. In this experiment, the effects of estradiol on extinction of a CTA was examined in 20 young (3 months) and 18 old (19 months) females. Young and old females were either untreated or treated with estradiol. Whereas, estradiol increased the extinction rate in young females it had no effect on extinction rate in old females. These results suggest that for CTAs, aging females have a reduced sensitivity to estradiol (Appendix C, presented at the American Psychological Society meeting, June, 1990).

Age-related changes in acquisition and extinction of conditioned taste aversions in males. There is an age-related

decrease in the sensitivity of the target tissues mediating sexual behavior to testosterone. The following experiments were designed to determine whether the same was true for target tissues mediating testosterone modulation of CTAs. Some investigators have reported prolonged extinction which is opposite of what one would expect in aged males with decreased testosterone levels. Thus in the first experiment, the extinction rates of 10 young (3 months) and 10 old (18 months) males were examined. The old males had slower extinction rates. In the second experiment, the acquisition rates of 40 young (3 months) and 40 old (18 months) males were examined. The young and old males were given 4 different doses of illness-inducing toxin. An analysis of the results indicates that old males do not acquire a CTA as readily as young males when low doses of LiCl are used. For those old and young males that acquired an aversion at a given dose, no differences in acquisition rate or extinction rate were found. These results suggest that old males have a lower sensitivity to LiCl than young males and that they and they differ from young males in how they process information about LiCl when one high dose is given but not when repeated low doses are given (Appendix G, to be presented at the American Psychological Association meeting, August, 1990; Appendix H, abstract submitted to the Gerontological Society).

Experimental Series 3: Fluid Deprivation Effects on Conditioned Taste Aversions

Effects of fluid deprivation on testosterone sensitivity and extinction. Fluid deprivation reduces behavioral sensitivity to testosterone (T). The amount of T required to prolong extinction of a conditioned taste aversion (CTA) is greater in fluid deprived than nondeprived male rats. In the first experiment, we observed that the minimum dose of T required to prolong extinction in fluid deprived Sprague-Dawley (SD) male rats is not sufficient in Fischer 344 (F344) males. To determine whether this is due to a greater effect of fluid deprivation on F344 males, 40 F344 and 40 SD male rats were either fluid deprived (1 hr/day access to fluid) or nondeprived (24 hr access to fluid). Following the first presentation of a 10% sucrose solution, a CTA was induced by injection of 0.15 M LiCl (10 ml/kg). Daily extinction trials began 2 days later and continued until criterion for extinction (100% of first day consumption) was reached. The fluid deprived F344 and SD rats extinguished faster than the nondeprived rats. However, the percentage increase in the extinction rates of the deprived F344 was 2-fold greater than that of the SD. These results suggest that F344 are affected more strongly by fluid deprivation and have a greater reduction in sensitivity to T (Appendix I, abstract submitted to Society for Neuroscience).

COMPUTATIONAL RESEARCH

We have completed a paper that lays the groundwork for developing a computational model for CTAs (Appendix J).

Background

The determination of the neural substrates for CTAs should involve the identification of four pathways: the US pathway, the CS pathway, the pathway for the elicited response to the CS prior to conditioning (UR_{CS} or unconditioned ingestive response, UIR), and the pathway for the elicited response to the CS after conditioning (CR_{CS} or unconditioned aversive response, UAR). Each taste is connected to both the ingestive and aversive patterns of responses. These connections are probably innate as hedonic reactions to taste have been observed in fetal and neonatal individuals (Pfaffman 1978, Steiner 1973, 1979).

The relative strengths of the two innate connections are dependent on the given taste. In the case of sucrose, the innate connection to the ingestive response is stronger than the innate connection to the aversive response. If exposure to sucrose is followed by illness, the connection to the ingestive response system will weaken and the connection to the aversive response system will strengthen. It is most likely that the illness-induced changes involve two rather than one process. Grill and Berridge (1985) have suggested that palatability processing involves two mechanisms and have provided evidence that the ingestive and aversive response systems can change independently. Thus, in order for the aversive response system to be expressed solely, a weakening of the ingestive response system would have to occur. If exposure to sucrose is not followed by negative consequences, a stronger connection to the ingestive response system will result. A stronger connection to the ingestive response system also will occur if a given taste is associated with positive reinforcement or if it is followed by recuperation from illness (Garcia et al 1977, Revusky 1967, 1974, Young 1966). So, experiential factors can alter the strengths of the innate connections to the ingestive and aversive response patterns. Thus, after a given taste is experienced, the relative strengths of the ingestive and aversive response systems are a function of the original innate connections, the number of exposures to sucrose with illness and the number of exposures to sucrose without illness. This hypothesis is supported by the findings that CTAs to nonpreferred tastes are stronger than to preferred tastes (Etscorn 1973), repeated pairings of a taste with illness strengthens an aversion and repeated pairings of a taste without illness reduces the strength of an aversion (Kalat & Rozin 1973).

There are other factors associated with the CS and US that can influence the strength of an aversion and therefore must be taken into account when developing a neural model for CTAs. The strength of an aversion has been found to be a function of the intensity of the taste as measured by concentration (Dragoin 1971) and the amount consumed on the first exposure (Bond & DiGuisto 1975), the intensity of the US (Revusky 1968) and prior experience with the US (Cannon et al 1975).

There are several factors which can modulate the development and strength of CTAs, but are not essential or critical for

aversion learning. The development and strength of an aversion is dependent on the hormonal milieu and deprivation state of the animal. The presence of testosterone (T) increases the proportion of animals that develop a CTA (Chambers et al 1981) and the presence of dexamethasone attenuates the strength of an aversion (Hennessy et al 1976). Water deprivation reduces the proportion of male rats that develop an aversion (Chambers et al 1981). It is interesting that deprivation can alter the hedonic value of tastes. Foods are reported to be more palatable with deprivation and less so with satiety (Cabanac 1971). Also, the number of ingestive responses decreases and the number of aversive responses increases as meal termination approaches (Grill & Berridge 1985). So, the relative strengths of the ingestive and aversive response systems are also a function of modulating factors. A complete understanding of the neural mechanisms controlling CTAs would include a determination of the neural circuitry for the modulating factors.

A neural model for extinction of a CTA can be outlined in a similar manner as acquisition. Extinction is a process by which connections to the aversive response system are weakened and connections to the ingestive response system are strengthened. Any information on the subsequent consequences of ingesting the CS is processed. If the consequences are neutral, that information serves to alter the relative strengths of the two response systems. Thus, after a CS has been experienced without negative consequences, the relative strengths of the ingestive and aversive response systems are a function of the relative strengths of these systems after the CTA, the number of exposures to the taste without illness, modulating factors, and probably the original innate predisposition.

Modeling Direction

Artificial neural network models can be placed in one of two categories: non real-time (spatial, static) and real-time (spatio-temporal) models. As temporal factors are critically important for classical conditioning, spatio-temporal models are being explored. We are examining the possibility that Klopff's drive-reinforcement (D-R) model can be adapted to the CTA problem (Klopff, 1988). We will begin to examine the case where a CTA is induced after repeated low doses of toxin are given. In this case acquisition of a CTA is similar to the typical S-shaped curve, although the S is inverted. We will then add a function that allows learning to take place more quickly as the strength of the toxin is increased.

To produce S-shaped acquisition curves, Klopff (1988) proposed that changes in synapse strength are proportional to the current synaptic strength at a given time. The mathematical specifications for the D-R model consist of two equations: an activation equation that calculates input/output signals on the basis of a weighted sum of input signals

$$y(t) = \sum_{i=1}^n w_i(t) x_i(t) - \theta$$

and a learning rule which determines changes in synapse strength due to changes in signal levels

$$\Delta w_i(t) = \Delta y(t) \sum_{j=1}^n c_j |w_i(t-j)| \Delta x_j(t-j)$$

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PUBLICATIONS

Chambers, K. C. A neural model for conditioned taste aversions; Annual Review of Neuroscience, 13, 373-385, 1990.

Chambers, K. C. and Yuan, D. Blockage of the effects of testosterone on extinction of a conditioned taste aversion by estradiol: Time of action. Physiology and Behavior, in press.

Sengstake, C. B. and Chambers, K. C. Sensitivity of male, female and androgenized female rats to testosterone during extinction of a conditioned taste aversion. Behavioral Neuroscience, in press.

MANUSCRIPTS IN PREPARATION

Yuan, D. and Chambers, K. C. Effects of estradiol on extinction of conditioned taste aversions. In Preparation.

Yuan, D. and Chambers, K. C. Changes in acquisition and extinction of conditioned taste aversions in aging male rats. In Preparation.

PRESENTATIONS AT MEETINGS

Yuan, D. and Chambers, K. C. Temporal analysis of estradiol blockage of testosterone effect on conditioned taste aversions. Poster presented at the first annual meeting of the American Psychological Society, Alexandria, VA, June 1989.

Yuan, D. L., Hung, C. and Chambers, K. C. Effects of gonadectomy on extinction of a conditioned food aversion. Abstract submitted for the Western Psychological Society meeting, Los Angeles, CA, April 1990.

Yuan, D. L. and Chambers, K. C. Age-related difference in the effect of estradiol on extinction of a conditioned food aversion. Poster presented at the second annual meeting of the American Psychological Society, Dallas, TX, June 1990.

PRESENTATION REQUESTS ACCEPTED

Yuan, D. L., Hung, C. and Chambers, K. C. Conditioned food aversion in young and old male rats. Poster to be presented at the American Psychological Association meeting, Boston, MA, August 1990.

PRESENTATION REQUESTS SUBMITTED

Brownson, E. A., Sengstake, C. B. and Chambers, K. C. Effects of fluid deprivation on testosterone sensitivity and extinction of a conditioned taste aversion. Society for Neuroscience meeting, St. Louis, October 1990.

Yuan, D. L., Hung, C. and Chambers, K. C. Failure to observe age-related differences in extinction of a conditioned food aversion when low doses of LiCl are used. Gerontological Society meeting, Boston, MA, November 1990.

APPENDIX A

Chambers, K. C. and Yuan, D. L. Blockage of the effects of testosterone on extinction of a conditioned taste aversion by estradiol: Time of action. Physiology and Behavior, in press.

Blockage of the Effects of Testosterone on Extinction of a Conditioned Taste Aversion by Estradiol: Time of Action

KATHLEEN C. CHAMBERS AND DAVID L. YUAN

Department of Psychology, University of Southern California

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CHAMBERS, K. C. AND D. L. YUAN. *Blockage of the effects of testosterone on extinction of a conditioned taste aversion by estradiol: Time of action.* PHYSIOL BEHAV 48(2) 000-000, 1990. — Testosterone (T) prolongs the extinction of a conditioned taste aversion only if it is present during extinction. Experiments were conducted to determine whether estradiol (E) blocks the effects of T by acting during acquisition or extinction. In the first experiment, gonadectomized male and female rats injected with estradiol dipropionate (EP) and testosterone propionate (TP) during extinction had significantly faster extinction rates than those only injected with TP. Treating gonadectomized rats with TP prior to as well as during extinction did not prevent EP from blocking the effects of T. In Experiment 2, E was equally effective in preventing T from prolonging extinction when it was implanted in gonadectomized males during acquisition, extinction, or both acquisition and extinction. Thus, E does not have to be present concurrently with T during extinction to be effective. This suggests that E does not act on a T-related mechanism but rather acts independently of T.

Conditioned taste aversion Estradiol Testosterone Acquisition Extinction

CONDITIONED taste aversions are extinguished more slowly in adult male than in adult female rats (6). The ability of males to show a prolonged extinction is dependent on the presence of testosterone (T). Gonadectomized males show a rapid rate of extinction which is not different than that of females whereas intact males and gonadectomized males administered testosterone propionate (TP) exhibit a slow extinction rate (2, 3, 10). Gonadectomy has no effect on the extinction rate of females. However, gonadectomized females will exhibit a slow extinction rate if they are administered TP. The effectiveness of testosterone in decreasing the rate of extinction to the same level as that of intact males or TP-treated gonadectomized males is influenced by the presence of the ovaries or of estradiol (2, 3, 11). Intact females treated with TP and gonadectomized females administered estradiol dipropionate (EP) and TP fail to show slower extinction rates than gonadectomized females only given TP.

Estradiol could block the effects of T by acting directly on a T-related mechanism or by acting independently of T. Testosterone prolongs extinction only when it is present during the extinction phase of the conditioned taste aversion (7). When gonadectomized female and male rats are administered TP during the acquisition phase of the conditioned taste aversion but not during the extinction phase, they show a rapid rate of extinction. However, when gonadectomized females and males are administered TP during extinction and either TP or vehicle during acquisition, they show a slow extinction rate. In order to under-

stand the mechanism by which E blocks the effects of T, it is necessary to determine when E is acting. Since in the previous studies E was administered with T during both acquisition and extinction, it is not known when E has its effects. The following experiments were conducted to determine whether E is blocking the effects of T during acquisition or extinction.

GENERAL METHOD

Subjects

The subjects were adult female and male Sprague-Dawley derived rats obtained from Simonson Laboratories. The rats were group housed from weaning until the start of the experiment. They then were housed one per cage in Experiment 1 and two per cage in Experiment 2. When housed two per cage, they were separated by a wood divider during conditioned taste aversion testing. The total time of separation was approximately 90 min. They were kept on ad lib food and fluids and a 12:12 hr light/dark cycle (lights off from 0100 hr to 1300 hr) throughout the experiment. Each day, water was available for 22 hr (Experiment 1) or 23 hr (Experiment 2) and either water or sucrose solution was available for the remaining 2 or 1 hr, respectively.

Surgeries and Hormonal Manipulations

In Experiment 1, gonadectomies were performed while the rats

¹Requests for reprints should be addressed to Kathleen C. Chambers, Department of Psychology, Seeley G. Mudd Building, University of Southern California, Los Angeles, CA 90089-1061.

were under sodium pentobarbital anesthesia (36 mg/kg of body weight for females and 48 mg/kg of body weight for males). In Experiment 2, gonadectomies and silastic capsule implantations were performed while the rats were under ether (Part A) or halothane (Part B) anesthesia. The hormones were administered either by injection or implantation. For injection, TP (1 mg/rat) and EP (150 µg/rat) were dissolved in 0.05 ml of sesame oil and injected SC at the nape of the neck. The selection of the doses of TP and EP was based on those used in previous studies (2,3). For implantation, silastic capsules (0.157-cm i.d. and 0.318-cm o.d.) were either unfilled or were filled with hormone and each end was sealed with silicone type A Silastic adhesive. Testosterone was placed in a 30-mm capsule and E was placed in a 5- or 10-mm capsule.

Conditioned Taste Aversion Procedure

The experimental procedure was divided into three periods: preconditioning, acquisition, and extinction. During all of these periods, the animals were weighed daily 1 hr before the end of the light part of the light/dark cycle. At the beginning of the dark part of the cycle, the water bottles of all animals were replaced with a cylinder of either chilled water or a chilled sucrose solution. The animals were given access to one of these solutions for 2 hr (Experiment 1) or 1 hr (Experiment 2) and then their water bottles were returned. All solutions were stored under refrigeration for 24 hr before they were given to the animals and were introduced at the beginning of the dark portion of the cycle.

During the preconditioning period, each animal was given chilled tap water. Animals housed two per cage also were adapted to the 90-min separation during this time. The acquisition period started immediately after termination of the preconditioning period. On acquisition day, all animals were given a cylinder of 10% (w/v; Experiment 1) or 9% (w/v; Experiment 2) sucrose solution. The amount of solution consumed was recorded for each animal, the sucrose solution was removed from the cage, and immediately thereafter the animal or animals in the cage were injected intraperitoneally with a 0.30 M LiCl solution (20 ml/kg). The extinction period began at least two days after acquisition. Chilled tap water was given during the interval between acquisition and extinction. During the extinction tests the sucrose solution was given but no LiCl injections were given. Animals were tested until they regained the consumption level of acquisition day or until a specified number of tests were given.

Bleeding and Hormone Assays

Blood was collected by tail vein under vacuum [no anesthesia (19)]. The total blood collection time from each rat was approximately 2 min. The samples were allowed to clot at 4°C for at least 4 hr. They then were centrifuged (5000 rpm/4000 × g for 20 min at 4°C) and serum was removed and stored in 0.5-ml portions at -20°C until assayed for E and T. The steroids were separated from the serum through extraction and chromatographic purification of LH-20 Sephadex columns and quantified by radioimmunoassay. For Experiment 1, the mean percentage of recovery, water blanks, and intraassay coefficients of variation were as follows: 69.8%, 5.4 pg, and 5.7%, respectively, for E and 86.5%, 1.3 pg, and 3.0%, respectively, for T. For Experiment 2, these values were as follows: 71.6%, 3.9 pg, and 12%, respectively, for E and 65.1%, 2.8 pg, and 9.6%, respectively, for T. Quantities calculated from standard curves were corrected for procedural losses and blank values.

Statistical Analyses

The number of days to extinction was computed for each

animal. For those animals that did not reach criterion, one plus the maximum number of extinction tests given was used as the extinction score. For independent groups designs, the hormonal values and extinction scores were analyzed with a two-factor or one-factor analysis of variance (ANOVA). The Newman-Keuls test was used to determine differences among pairs of treatments. In cases where there was no variance in one of the groups, the nonparametric tests, Kruskal-Wallis and Conover Multiple Comparison (8), also were used. For the repeated measures design, a two-factor ANOVA with repeated measures on one factor was used. Single factor ANOVAs with repeated measures on one factor were used to determine whether each sex differed across time, *t*-tests were used to determine sex differences at each time, and planned comparisons were used to determine differences between time 1 and times 2 and 3.

EXPERIMENT 1

In this experiment, E was given only during extinction to determine whether it could block the effects of T during extinction. Testosterone was given only during extinction in part A of this experiment and it was given prior to as well as during extinction in part B.

Part A: Method

Twenty-four females and 24 males (77–168 days old) were assigned in equal numbers to one of four groups: 1) oil during acquisition and extinction (O/O); 2) TP during acquisition and extinction (T/T); 3) EP and TP during acquisition and extinction (ET/ET); 4) oil during acquisition and EP and TP during extinction (O/ET). All rats were gonadectomized 3 weeks before the experiment began. Injections of vehicle and hormone were given daily 1 hr before the end of the light part of the light/dark cycle. The preconditioning period and the injections for the acquisition period were initiated 7 days before acquisition. The injections were continued daily until 20 days after acquisition. On this day, the injections for the extinction period were initiated and were continued daily until the end of the experiment. Seven days later, extinction tests began and were continued until criterion was reached or until 42 tests were given.

An additional 6 females and 6 males were bled two weeks after gonadectomy. Injections of EP and TP were initiated one week after bleeding and were continued for 9 days. The rats were bled again 1 and 33 days after the last injection.

Behavioral Results

The data from one female injected with EP and TP during acquisition and extinction were eliminated from any analyses because she died before the experiment was completed. The 3 groups of rats did not differ significantly in the amount of sucrose consumed on the day of acquisition or on the first day of extinction. Estradiol prevented T from prolonging extinction in both females and males when it was administered during extinction (Fig. 1). The extinction rates of the males and females did not differ following any of the hormonal treatments. The T/T males and females had significantly slower extinction rates than the other 3 groups and the O/ET males and females had slower rates than the O/O or ET/ET males and females [$F(3,135) = 36.35$, $p < 0.0001$, hormone treatment main effect; Newman-Keuls test, $p < 0.05$]. The extinction rates of the O/O and ET/ET males and females did not differ significantly.

Hormonal Results

The E levels of the males and females did not differ. Mean

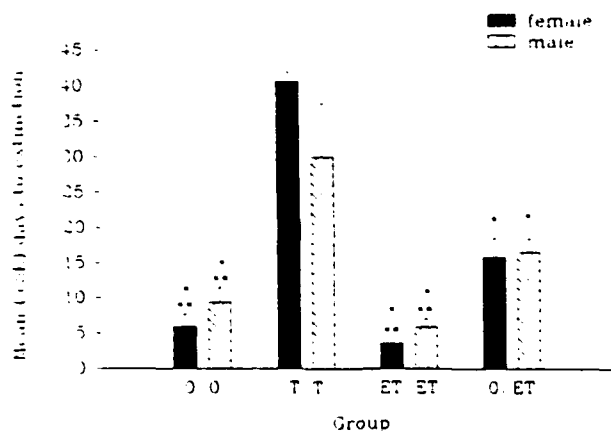


FIG. 1. For Experiment 1A, mean (\pm SE) days to extinguish a conditioned taste aversion in gonadectomized female and male rats given oil (O), testosterone propionate (T), or estradiol dipropionate (E) plus T (ET). Animals were given these treatments during acquisition (before slash) or during extinction (after slash). *Significantly different than the T/T females and males, $p < 0.05$. **Significantly different than the O/ET females and males, $p < 0.05$.

(\pm SE) estradiol levels for males and females rose from 27.7 (\pm 4.3) pg/ml to 2425.0 (\pm 69.4) pg/ml after daily injections of EP and TP, $F(1,10) = 1281.73$, $p < 0.0001$. Estradiol levels were still elevated 33 days after injections were terminated [mean (\pm SE) = 94.33 (\pm 11.58) pg/ml; $F(1,10) = 33.96$, $p < 0.001$].

The T levels of the males and females differed significantly [$F(1,10) = 7.31$, $p = 0.02$, sex main effect, and $F(2,20) = 9.46$, $p = 0.001$, sex by hormone treatment interaction]. For both males and females, T levels rose significantly after daily injections of EP and TP [from means (\pm SE) of 0.13 (\pm 0.04) and 0.16 (\pm 0.06) ng/ml, respectively, to 25.50 (\pm 3.13) and 35.95 (\pm 1.66) ng/ml, respectively; $F(1,5) = 66.82$ and 468.33, respectively, $p < 0.001$] and T levels were still elevated 33 days after injections were terminated [0.88 (\pm 0.15) and 0.31 (\pm 0.04) ng/ml, respectively; $F(1,5) = 31.94$ and 7.65, respectively, $p < 0.05$]. However, the T levels of the females were significantly higher than those of the males 1 day after termination of the injections, $t(10) = 2.95$, $p < 0.02$, but were significantly lower 33 days after injections were terminated, $t(10) = 3.68$, $p < 0.01$.

Part B: Method

Thirty-six males (66–143 days old) were assigned in equal numbers to one of six groups: 1) oil during acquisition and extinction and no treatment during the postacquisition/preextinction period (O/N/O); 2) TP during acquisition and extinction and no treatment during the postacquisition/preextinction period (T/N/T); 3) EP and TP during acquisition and extinction no treatment during the postacquisition/preextinction period (ET/N/ET); 4) TP during acquisition, no treatment during the postacquisition/preextinction period, and EP and TP during extinction (T/N/ET); 5) oil during acquisition, TP during the postacquisition/preextinction period, and EP and TP during extinction (O/T/ET); 6) oil during acquisition, no treatment during the postacquisition/preextinction period, and EP and TP during extinction (O/N/ET). All rats were gonadectomized 2–6 weeks before the start of the experiment. Injections of vehicle and hormone were given daily 1 hr before the end of the light part of the light/dark cycle. The preconditioning period and the injections for the acquisition period were initiated 7

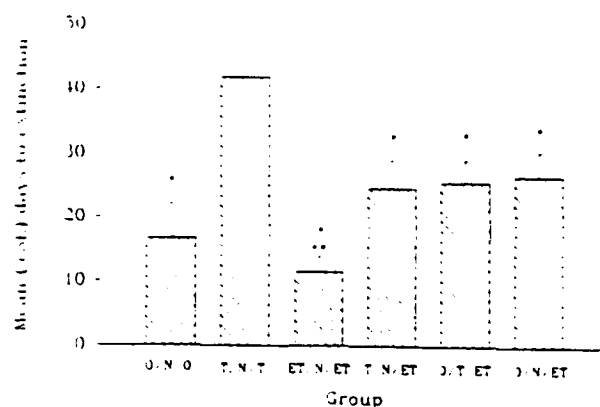


FIG. 2. For Experiment 1B, mean (\pm SE) days to extinguish a conditioned taste aversion in gonadectomized male rats given oil (O), testosterone propionate (T), estradiol dipropionate (E) plus T (ET), or no treatment (N). Animals were given these treatments during acquisition (before first slash), during the postacquisition/preextinction period (after first slash and before second slash), and during extinction (after both slashes). *Significantly different than T/N/T, $p < 0.05$. **Significantly different than T/N/ET, and O/N/ET, $p < 0.05$.

days before acquisition. The injections continued daily until two days after acquisition. On the third day after acquisition and continuing for 6 days, the injections for the postacquisition/preextinction period were given. On the day following the end of this period, injections for the extinction period were initiated and were continued until the end of the experiment. Extinction tests began 7 days after the injections were initiated. The tests were given daily until criterion was reached or until 42 tests were given.

Results

The 6 groups of males did not differ significantly in the amount of sucrose consumed on the day of acquisition or on the first day of extinction. The effects of E on extinction rate were not attenuated when T was administered prior to as well as during extinction (Fig. 2). The T/N/T males had slower extinction rates than the other 5 groups of males [$\chi^2(5) = 19.77$, $p = 0.0014$; Conover Multiple Comparisons, $p < 0.05$, and $F(5,30) = 7.94$, $p < 0.0001$; Newman-Keuls test, $p < 0.05$]. The extinction rates of the T/N/ET, O/T/ET, and O/N/ET males were significantly slower than those of the ET/N/ET males but not the O/N/O males (Conover Multiple Comparisons, $p < 0.05$ and Newman-Keuls T test, $p < 0.05$). The extinction rates of the T/N/ET, O/T/ET, and O/N/ET males did not differ and those of the ET/N/ET and O/N/O did not differ.

EXPERIMENT 2

The results of Experiment 1 clearly indicate that the presence of E during extinction can partially block the effects of T. The presence of E during both acquisition and extinction, however, was more effective than its presence only during extinction. This suggests that E may also act during acquisition. Using EP to test this suggestion, however, is problematic since E in this form is long acting. Estradiol levels were still elevated 33 days after termination of EP injections. Legan, Coon and Karsch (16) have found that when E is administered to gonadectomized rats by implanting silastic capsules filled with E, serum E levels return to baseline levels within two hours after removal of the capsules. This suggests that administration of E by capsule is a viable means

by which to assess the role of E in acquisition. Thus, in Part A of this experiment, different dosages of E first were administered by capsule during both acquisition and extinction to determine a dosage that was effective in preventing T from prolonging extinction. Then, in Part B, the effective dosage of E was administered by capsule during acquisition or during extinction to determine whether it would block the effects of T when present during acquisition as well as extinction.

Part A: Method

Thirty-two males (180 days old) were randomly assigned in equal numbers to one of four groups: 1) 10-mm and 30-mm blank capsules (0); 2) 30-mm T capsules (T); 3) 5-mm E and 30-mm T capsules (5ET); 4) 10-mm E and 30-mm T capsules (10ET). The preconditioning period was initiated three days after all rats were gonadectomized and implanted. Ten days after surgery, acquisition was induced and two days after acquisition, extinction tests were initiated. Tests were given until criterion was reached or until 45 tests had been given.

Results

The data from 7 rats (2 from the 0, 2 from the T, and 3 from the 10ET groups) were eliminated from any analyses because these rats drank less than 2 ml of sucrose solution on the day of acquisition. The four groups of males did not differ significantly in the amount of sucrose consumed on the day of acquisition or on the first day of extinction. A 10-mm E capsule but not a 5-mm capsule was effective in preventing T from prolonging extinction. The extinction rates of the T and 5ET groups were significantly slower than those of the 0 and 10ET groups [$F(3,21)=3.81$, $p=0.025$; Newman-Keuls test, $p<0.05$]. The extinction rates of the T and 5ET groups did not differ and those of the 0 and 10ET groups did not differ.

Part B: Method

Fifty males (100 days old) were randomly assigned in equal numbers to one of five groups: 1) blank capsules in both acquisition and extinction (0/0); 2) T capsules in both acquisition and extinction (T/T); 3) E and T capsules in both acquisition and extinction (ET/ET); 4) T capsules in acquisition and E and T capsules in extinction (T/ET); 5) E and T capsules in acquisition and T capsules in extinction (ET/T). The blank capsules were 10-mm and 30-mm long, the T capsules were 30-mm long, and the E capsules were 10-mm long. The preconditioning period was initiated three days after all rats were gonadectomized and implanted. Nine days after surgery, acquisition was induced. Two days later, the capsules for acquisition were replaced in each group. Extinction tests began 9 days after capsule replacement. Tests were given until criterion was reached or until 30 tests were given. Blood samples were taken from all rats 2 days after the 30th test.

Behavioral Results

The data from 1 rat (ET/ET) were eliminated from any analyses because of an incorrect capsule placement. The five groups of males did not differ significantly in the amount of sucrose solution consumed on the day of acquisition or on the first day of extinction. Estradiol prevented T from prolonging extinction when it was administered during acquisition (Fig. 3). The 0/0, ET/ET, ET/T, and T/ET groups extinguished faster than the T/T group [$F(4,44)=5.25$, $p=0.0015$; Newman-Keuls test, $p<0.05$]. The

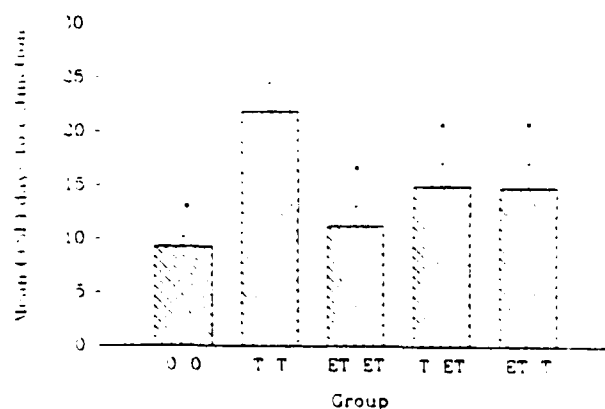


FIG. 3. For Experiment 2B, mean (\pm SE) days to extinguish a conditioned taste aversion in gonadectomized male rats implanted with blank (0), testosterone-filled (T), or estradiol-filled (E) and T-filled (ET) capsules. Animals were given these treatments during acquisition (before slash) or during extinction (after slash). *Significantly different than T/T, $p<0.05$.

extinction rates of the 0/0, ET/ET, ET/T, and T/ET groups did not differ.

Hormonal Results

The E data from 1 rat (0/0) were lost. Those males with E capsules during extinction had significantly higher E levels than those without E capsules [$F(4,41)=7.85$, $p<0.0001$; Newman-Keuls test, $p<0.05$]. The E levels of the ET/ET [mean (\pm SE) = 101.5 (\pm 13.9)] and T/ET [mean (\pm SE) = 114.4 (\pm 21.6)] groups did not differ and those of the 0/0 [mean (\pm SE) = 35.8 (\pm 5.3)], T/T [mean (\pm SE) = 51.3 (\pm 10.0)], and ET/T [mean (\pm SE) = 45.7 (\pm 7.0)] groups did not differ. The T levels of the four groups of males that had T capsules during extinction, the T/T [mean (\pm SE) = 3.08 (\pm 0.10)], ET/T [mean (\pm SE) = 3.45 (\pm 0.18)], T/ET [mean (\pm SE) = 3.35 (\pm 0.09)], and ET/ET [mean (\pm SE) = 3.38 (\pm 0.20)] groups, did not differ but were higher than those of the males with blank capsules [means (\pm SE) = 0.16 (\pm 0.04) ng/ml, respectively; $F(4,42)=119.38$, $p<0.001$; Newman-Keuls test, $p<0.05$].

GENERAL DISCUSSION

Estradiol was effective in blocking the longer extinction rates induced by T when it was administered during acquisition, extinction, and both acquisition and extinction. In Experiment 2, E was equally effective when administered during each of these time periods. However, in Experiment 1, E was more effective when given during both acquisition and extinction than when given only during extinction. The reason for this discrepancy is unclear although it may be associated with the differences in the doses of E or T used in the two experiments. The blood levels of E were 22 times higher in Experiment 1 than Experiment 2 and the levels of T were 9 times higher. Earley and Leonard (11) have suggested that the ratio of estrogen to androgen rather than either hormone alone is the crucial factor in determining the rate of extinction. But the differences in the results of Experiments 1 and 2 cannot be accounted for by differences in the ratios of E to T since the ratio was higher in Experiment 1 than in Experiment 2.

Testosterone is effective in slowing extinction of a conditioned taste aversion in gonadectomized rats when it is present during extinction but not when it is present during acquisition (7). The results of Experiments 1 and 2 clearly show that E does not have

to be present concurrently with T during extinction to be effective since E prevents T from prolonging extinction when it is administered during acquisition or during extinction. This suggests that E does not act on a T-related mechanism but rather acts independently of T.

If E acts independently of T, then one would expect that E would increase extinction rate regardless of the presence of T during extinction. Although we have not found significant increases in extinction in gonadectomized males treated with E alone, there were tendencies for these males to extinguish faster (3). Also, Earley and Leonard (11) have found that when gonadectomized males were given a preexposure to sucrose 1 day before conditioning, E-treated males extinguished faster than vehicle-treated males.

A number of different hypotheses have been proposed to account for the effects of T on extinction of a conditioned taste aversion, e.g., T decreases relearning, retards forgetting, or increases persistence behavior (4, 7, 10). Although E may also affect these processes, it is more likely that it affects processes that are common to both acquisition and extinction. Estrogen is known to produce nausea and vomiting in humans (12). Recently, it has been reported that E can be used as a toxin to induce a conditioned taste aversion in gonadectomized male and female rodents (13, 17). It is possible that E prevents T from prolonging extinction because of its toxic effect. A number of investigators have shown that preexposure to a toxin before acquisition or extinction can attenuate the subsequent learning or accelerate the subsequent extinction, respectively, of an aversion (1, 9, 14, 18, 20, 21). In our studies, E was injected or implanted several days before acquisition

or extinction. If the effect of E is mainly a toxic one, the increased rate of extinction that we found is consistent with the results described in the studies above. However, it has not been established whether physiological levels of E are effective in producing conditioned taste aversions. Estradiol levels that are within the physiological range can effectively block the effects of T. For example, T is not effective in prolonging extinction in intact females (2). In intact females, the mean (+SE) levels of E range from 28.0 (+1.4) pg/ml to 81.0 (+5.4) pg/ml during the estrous cycle (15). Fifty-three percent of the ET-treated males in Experiment 2 had E levels that were the same or lower than the mean level found in intact females. Although all of the ET-treated males had E levels that were higher than what we have found in young intact males (range of 16–43 pg/ml), 50% of the males had E levels within the range observed in old intact males (19–113 pg/ml) (5). This suggests that, at least for some males, E levels that are within the physiological range for intact females and aged males can be effective in blocking the effects of T. Thus, physiological levels of E are an important aspect of any hypothesis proposed to account for how E prevents T from prolonging extinction of a conditioned taste aversion.

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APPENDIX B

Temporal Analysis of Estradiol Blockage of Testosterone
Effect on Conditioned Taste Aversions

David L. Yuan and Kathleen C. Chambers
Department of Psychology
University of Southern California
Los Angeles, CA 90089-1061

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Adult male rats exhibit stronger resistance to extinction than adult female rats in the conditioned taste aversion (CTA) paradigm (Chambers & Sengstake, 1976). After males are gonadectomized, their extinction rate is not different from that of intact females. Gonadectomized females show no change in extinction rate. When gonadectomized males are given testosterone (T) replacement, the rate of extinction is similar to that of intact males (Chambers, 1976). Thus T mediates the slower rate of CTA extinction observed in male rats.

Although T treatment is effective in slowing extinction in gonadectomized females it has a diminished effect in intact females (Chambers, 1976). When estradiol (E) and T are given to gonadectomized females the effectiveness of T in prolonging extinction also is reduced (Chambers, 1980). This suggests that E from the ovaries diminishes the effect of T on the rate of CTA extinction in intact females.

E could block the T-induced slow extinction rate by acting directly on a T-related mechanism or by acting independently of T. In order to understand the mechanism by which E blocks the effects of T, it is necessary to determine the following:

1. When during the CTA process does E act to block the T effect?
2. Does E have an effect on extinction when it is given alone?

EXPERIMENT 1

T acts to prolong extinction only if it is present during the extinction phase of the CTA (Chambers & Sengstake, 1979). It is not effective if it is present only during acquisition. If E acts on a T mechanism then E should be effective if it is present during extinction but not if it is present during acquisition. This experiment was designed to test whether E has the same time course of action as T.

METHOD

Design. 50 adult male rats were randomly assigned to one of the following five groups:

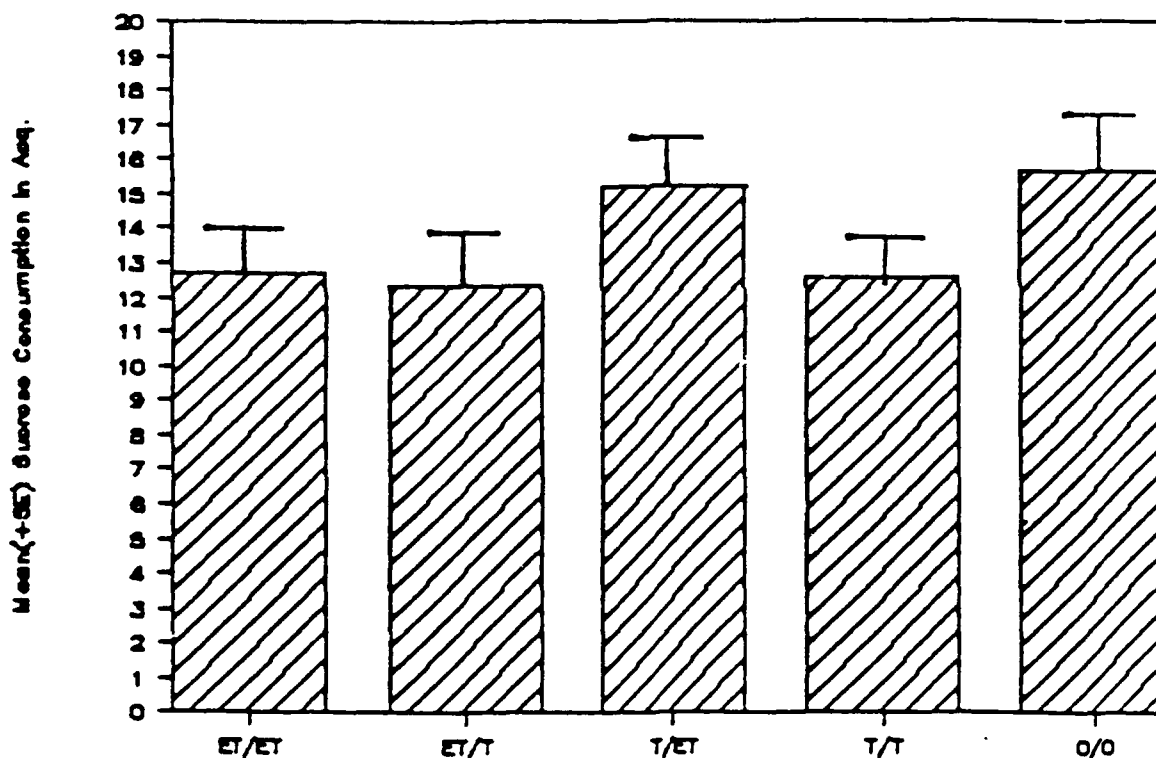
- | | |
|-------|---|
| 0/0 | No hormone in both acquisition and extinction |
| ET/ET | E and T in both acquisition and extinction |
| T/T | T in both acquisition and extinction |
| T/ET | T in acquisition and E and T in extinction |
| ET/T | T and E in acquisition and T in extinction |

CTA Procedure. All of the males were gonadectomized and hormones were administered through subcutaneously implanted silastic capsules. Nine days after surgery, the acquisition phase of the CTA procedure was initiated. All of the males were given a 9% chilled sucrose solution for one hr and then were injected ip with a 0.30 M LiCl solution (20cc/kg body weight). Two days later the acquisition phase hormone implants were replaced with the extinction phase implants. Nine days later the extinction phase was initiated. Starting on this day and continuing for the next 46 days, the males were given 9% sucrose solution for one hr.

RESULTS

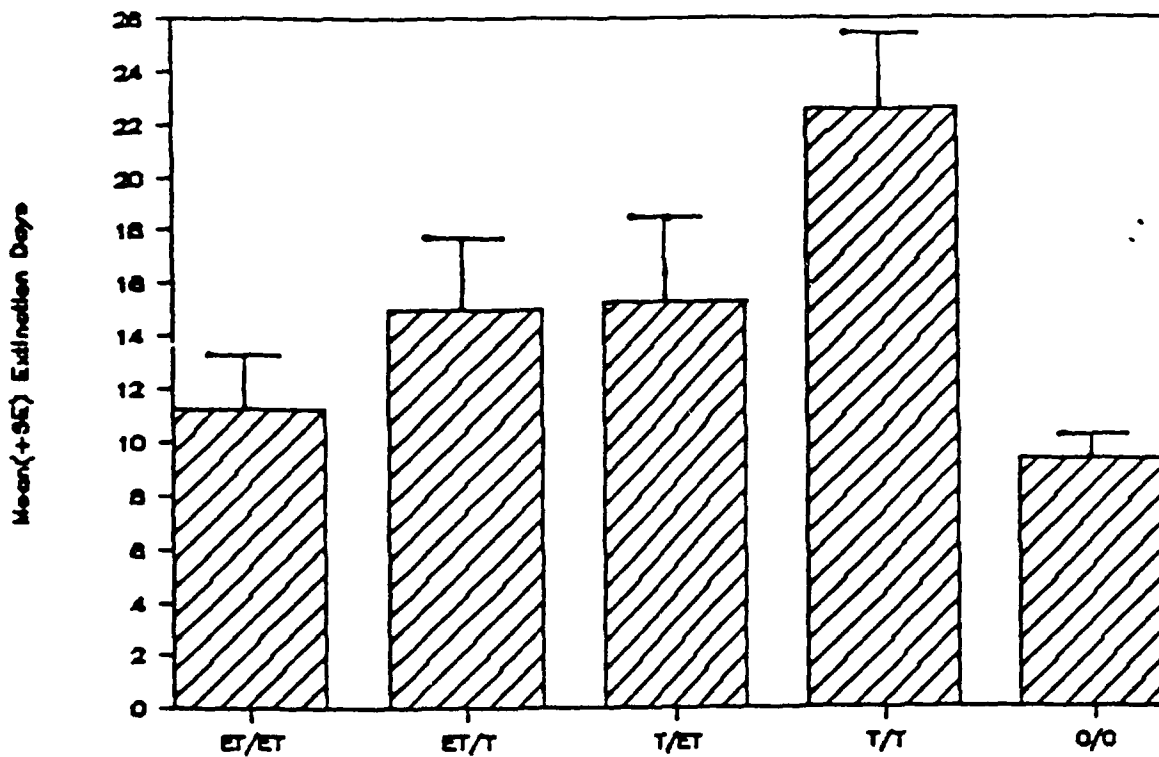
The five groups of males did not differ in the amount of sucrose consumed on the day of acquisition (Figure 1).

Figure 1



Extinction rates (the number of days to reach 100% of acquisition day consumption) were computed for each male. The extinction rates of the five groups differed significantly ($F[4,44]=5.25$, $p=0.0015$). The Waller-Duncan K-ratio T test indicated that the 3 groups with estradiol (ET/T, T/ET and ET/ET) and the no hormone group (O/O) did not differ but these four groups extinguished faster than group T/T (Figure 2).

Figure 2



CONCLUSION

The results of this experiment show that E can act either during acquisition or extinction.

EXPERIMENT 2

Although we previously found that E itself had no significant effect on extinction in gonadectomized males and females, the number of animals tested was small and the extinction rates tended to be lower than those of gonadectomized controls (Chambers, 1980). Also, Earley and Leonard (1979) reported that when gonadectomized males were given one or two preconditioning exposures to the sucrose solution prior to CTA induction, E increased the extinction rate. The following study was designed to determine whether E increases the rate of extinction of a CTA.

METHOD

Design. 30 adult female rats were randomly assigned to one of the following groups:

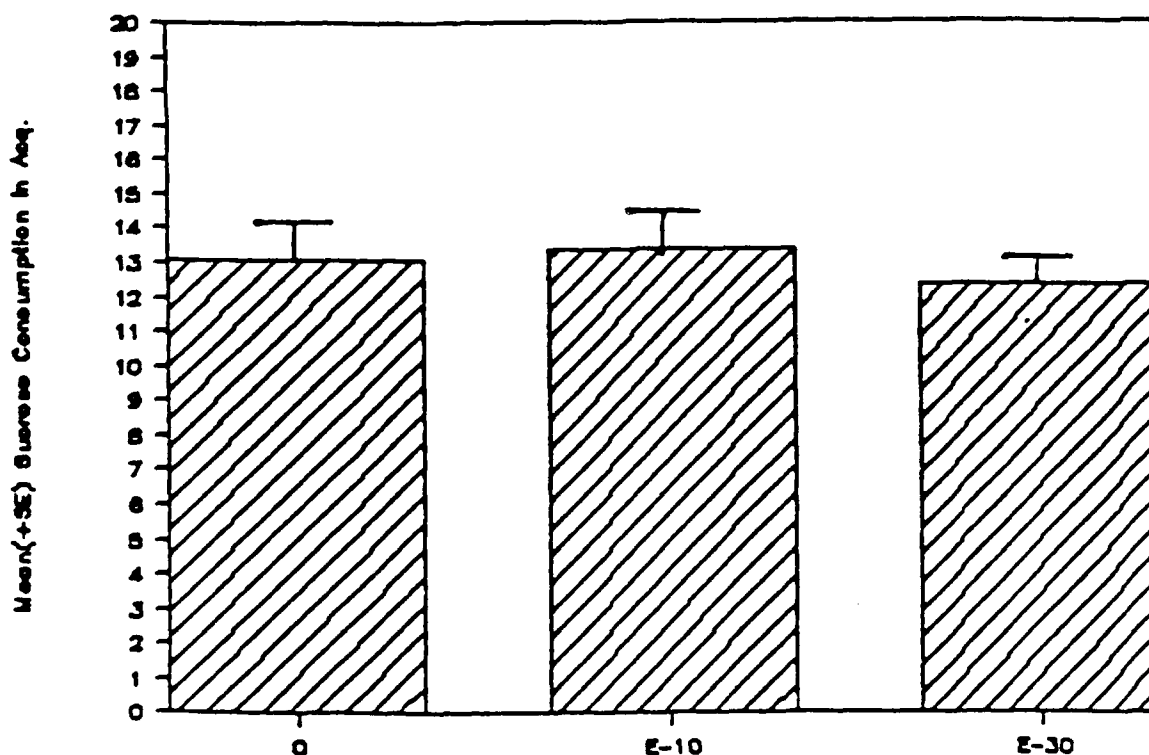
- 0 No hormone in acquisition and extinction
- E-10 Low E dose in acquisition and extinction
- E-30 High E dose in acquisition and extinction

CTA Procedure. All of the females were gonadectomized and E was administered through subcutaneously implanted silastic capsules. Twelve days after surgery, the acquisition phase of the CTA procedure was initiated. All of the females were given a 9% chilled sucrose solution for one hr and then were injected ip with a 0.30 M LiCl solution (20cc/kg body weight). Two days later the extinction phase was initiated. Starting on this day and continuing for the next 20 days, the females were given 9% sucrose solution for one hr.

RESULTS

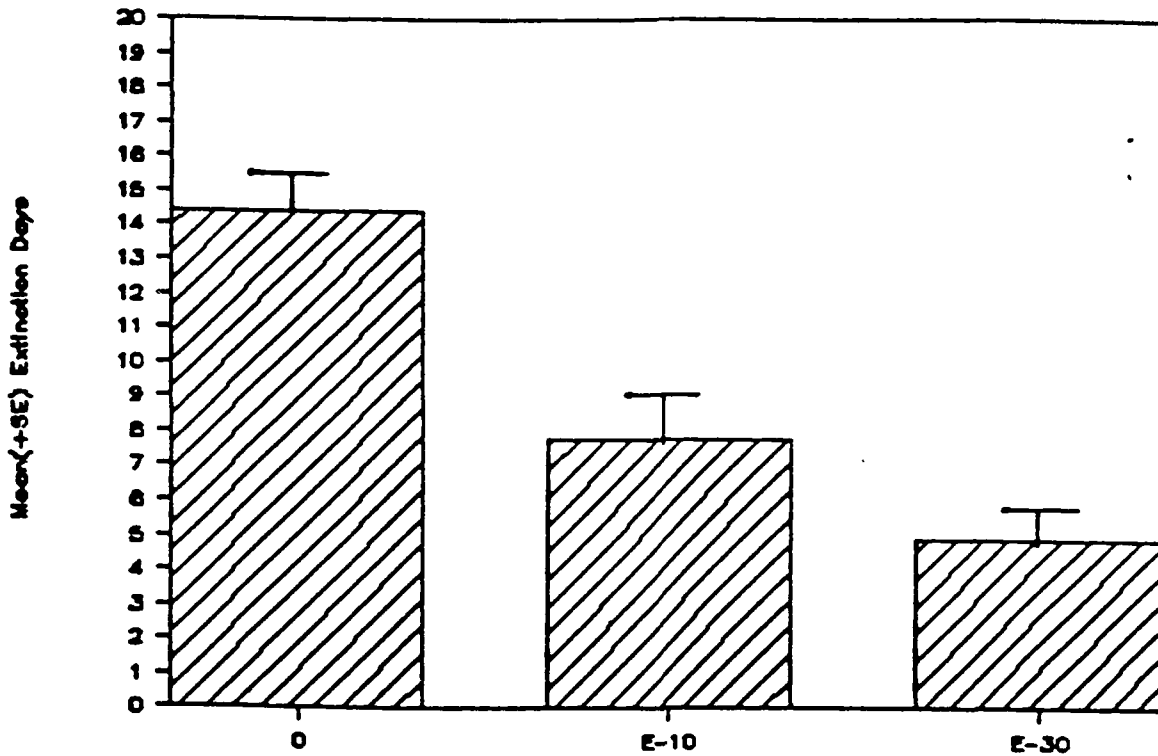
The three groups of females did not differ in the amount of sucrose consumed on the day of acquisition (Figure 3).

Figure 3



Extinction rates (the number of days to reach 100% of acquisition day consumption) were computed for each female. The extinction rates of each of the three groups differed significantly ($F[2,27]=23.45$, $p<0.0001$; Waller-Duncan K-ratio T test; Figure 4).

Figure 4



CONCLUSION

The results of this experiment show that E has an effect on CTA extinction when it is given alone.

GENERAL CONCLUSIONS

The results of these experiments suggest that E does not act on a T-related mechanism but rather acts independently of T.

Recently, Gustavson and Gustavson (1987) reported that E can be used as a toxin to induce a CTA. We suggest that the E blockage of the T-induced slow extinction can be explained in terms of its toxic effects.

Cannon, Berman, Baker and Atkinson (1975) have shown that presentation of one toxin that is not paired to a food before acquisition or extinction of a CTA induced by another toxin can attenuate the acquisition and extinction processes.

ACKNOWLEDGEMENT

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APPENDIX C

Age-related difference in the effect of estradiol on extinction
of a conditioned food aversion

David L. Yuan and Kathleen C. Chambers

Poster presented at the second annual meeting of the
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Dalles, TX
June 1989

AGE-RELATED DIFFERENCE IN THE EFFECT OF ESTRADIOL ON EXTINCTION OF A CONDITIONED FOOD AVERSION

David L. Yuan and Kathleen C. Chambers

Department of Psychology, USC, Los Angeles, CA 90089

Abstract

The effect of estradiol on extinction of a conditioned food aversion in old and young ovariectomized rats was examined. Estradiol increased the extinction rates in young but not old females.

Introduction

We have shown that when estradiol and testosterone are administered to gonadectomized male and female rats, estradiol prevents testosterone from prolonging extinction of a conditioned food aversion. In addition, when estradiol is administered alone, it increases the rate of extinction. Aging females are less sensitive to the stimulating effects of estradiol on reproductive behavior (Peng, Chuong & Peng, 1977; Wise & Parsons, 1984). This change may be specific to pathways mediating this behavior or may be more general. The following experiment was designed to determine whether there is a change in the sensitivity to the effects of estradiol on extinction of a conditioned food aversion.

Methods

Twelve old (20 months) and 16 young (3 months) Fischer 344 female rats were randomly assigned to one of two groups: empty capsule or estradiol-filled capsule.

All of the females were ovariectomized and implanted subcutaneously with either empty or estradiol-filled capsules. The rats then were housed two per cage and were kept on a 12:12 hr light/dark cycle.

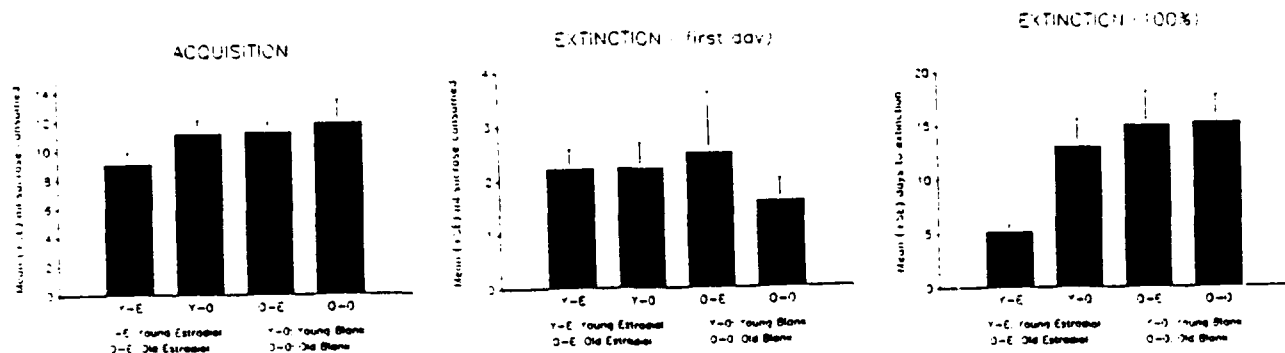
Eleven days after surgery a conditioned food aversion was induced. On the day of acquisition the rats were given access to a chilled 9% sucrose solution (wt/vol in water) at the beginning of the dark phase of the light/dark cycle. One hour later, they were injected with LiCl (0.15 M, 10 ml/kg). Daily extinction tests were initiated two days later. During these tests the rats were given a 9% sucrose solution for one hr but no LiCl injections were given. Animals were

tested until they regained the consumption levels of acquisition day or until 28 tests had been given.

Results

The four groups of females did not differ significantly in the amount of sucrose consumed on the day of acquisition or on the first day of extinction.

The extinction rates of young estradiol-implanted females were significantly faster than the old estradiol-implanted females and the old and young untreated females ($F[3,24] = 3.55$, $p < .05$; Newman-Keuls test, $p < .05$). The extinction rates of the latter three groups were not different.



Conclusion

These results strongly suggest that aged females have a reduced sensitivity to the effects of estradiol on extinction of a conditioned food aversion as well as reproductive behavior.

The mechanism by which estradiol accelerates extinction is unknown. But it is likely that aging causes a deterioration in the system mediating this effect.

Decreases in reproductive behavior of aged females are associated with decreases in uterine and brain nuclear estrogen receptors (Han, Kokkonen & Roth, 1989; Wise & Parsons, 1984). This suggests that the age-related decreases in reproductive behavior are due to loss of estrogen receptors. Loss of estrogen receptors also may account for the inability of estradiol to accelerate extinction of conditioned food aversions in aged females.

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APPENDIX D

Preliminary Study

Effects of amygdala lesions on acquisition of conditioned taste aversions

Elizabeth A. Brownson, Richard F. Thompson,
and Kathleen C. Chambers

Departments of Biological Sciences and Psychology
University of Southern California

Several investigators have found that lesions of the amygdala, in particular the basolateral amygdaloid nucleus, disrupt acquisition and retention of prelesion conditioned taste aversions (Aggleton et al., 1981; Mikulka et al., 1977; Nachman and Ashe, 1974; Simbayi et al., 1986). But after finding that cutting the connections between the amygdala and the temporal cortex produced the same deficits as lesions of the basolateral amygdala, Fitzgerald and Burton (1983) suggested that it is the destruction of the fibers of passage that produces the deficits after lesions of the basolateral amygdala and not the destruction of the nucleus itself. Recently, Dunn and Everitt (1988) found that neurotoxic (ibotenic acid) lesions which spare fibers of passage had no effect on aversion learning whereas electrolytic lesions which destroy both cells and fibers attenuated the aversion. Neither neurotoxic or electrolytic lesions had a significant effect on extinction. They suggested that it is the axons passing between the brain stem/hypothalamus and gustatory neocortex that are responsible for the deficits after electrolytic lesions of the amygdala.

The role of the amygdala in conditioned taste aversions was of particular interest to us because of its apparent involvement in hormonally mediated behaviors and because of the presence of androgen and estrogen receptors (Sar & Stumpf, 1973; Stumpf & Sar, 1978). However, in light of this evidence, it seems unlikely that it mediates the effects of gonadal hormones on conditioned taste aversions. Thus, we set out to confirm and extend these findings. The following studies were designed specifically to determine the effects of electrolytic and neurotoxic lesions on acquisition of an aversion induced after lesioning and acquisition of an aversion induced before lesioning.

Method

Subjects. The subjects were 80 Fischer 344 male rats that were 102-117 days old and weighed 295-310 gm. The males were group housed from the time of weaning and were housed 2 per cage during the experiment. They were separated by a stainless steel divider during conditioned taste aversion testing. They were kept on ad lib food and fluids and a 12:12 hr light/dark cycle (lights off at 1800 hr). Each day, water was available for 23 hr and either water or sucrose solution was available for the remaining 1 hr, respectively. The males were randomly divided into 4 groups: nonsurgery control (CONT), sham control (SHAM), electrolytic lesion (ELEC), and neurotoxic lesion (NMDA). For half of the males, a conditioned taste aversion was induced before surgery (Acquisition-Before) and for the other half it was induced after surgery (Acquisition-After).

Surgery. Under nembutal anesthesia, the males were placed in a Kopf stereotaxic device, the scalp retracted, and the skull trephined. For the neurotoxic lesion a cannula (30 gauge stainless steel) was inserted, for the electrolytic lesion an electrode insulated except at the tip (00 gauge stainless steel, 0.5 mm exposed tip) was inserted, and for the sham control a similar electrode was inserted. Lesions were made at the following coordinates (based on Paxinos and Watson, 1986; incisor bar set at -3.3 mm): 2.9 mm posterior to bregma, lateral +/-5.0 mm, ventral (from dura) -8.0 mm. The neurotoxic lesion (N-methyl-D, L-aspartic acid, NMDA; 12 ug/0.6 ul normal saline) was injected over a 2-3 min period. To avoid the spread of toxin along the shaft, the cannula was withdrawn after a period of at least 5 min and the amount of time taken to withdraw the cannula was at least 2 min. The electrolytic lesion was made by a Grass DC constant current lesion maker set at 0, turned to 2 mamps in 4 sec, and kept at 2 mamps for 30 sec.

Conditioned Taste Aversion Procedure. The experimental procedure was divided into three periods: preconditioning, acquisition, and extinction. During all of these periods, the animals were weighed daily 1 hr before the end of the light part of the light/dark cycle. At the beginning of the dark part of the cycle, the water bottles of all animals were replaced with a cylinder of either chilled water or a chilled sucrose solution. The animals were given access to one of these solutions for 1 hr and then their water bottles were returned. All solutions were stored under refrigeration for 24 hr before they were given to

the animals.

The Acquisition-Before males were given preconditioning and acquisition trials when 101-114 days old, underwent surgery 3 days after acquisition, and were given a retention test 23 days after surgery. The Acquisition-After males underwent surgery when 101-114 days old, were given an acquisition trial 23 days after surgery, and were given a retention test 2 days after acquisition. For all males, the preconditioning period lasted 7 days. Each animal was given chilled tap water for 1 hr. The acquisition period started immediately after termination of the preconditioning period. On acquisition day all animals were given a cylinder of 10% (w/v) sucrose solution for 1 hr. The amount of solution consumed was recorded for each animal and the animals were injected with a 0.15 M LiCl solution (10 ml/kg). The retention test involved giving the males access to the sucrose solution but not giving them LiCl injections.

Histology. About 2 weeks after behavioral testing the males were deeply anesthetized with nembutal, their brains were removed and post-fixed in a 30% sucrose-formalin solution for at least 48 h prior to sectioning. Sections were cut at 50 micron section thickness on a sliding microtome. Every second section was mounted on a glass slide and stained with Cresyl Violet. The electrolytic lesions were assessed by directly observing the limits of the lesion in the anterior-posterior, dorsal-ventral, and medial-lateral planes. The NMDA lesions were assessed by demarcating the absence of nerve cell bodies and the presence of areas of obvious intense gliosis.

Results

Four CONT and one SHAM Acquisition-Before males were eliminated from all analyses because of insufficient sucrose consumption (less than 3 cc). Four ELEC and 1 NMDA Acquisition-Before and 2 ELEC and 5 NMDA Acquisition-After males were eliminated from all analyses because of insufficient sucrose consumption, deaths, or histological reasons.

Histology. The ELEC animals sustained complete bilateral damage of the anterior, posterior and ventral aspects of the basolateral amygdala region throughout the anterior-posterior extent (see Figure 1). Typically the lesions were large and damage was sustained to other adjacent areas. Areas frequently lesioned included the basomedial nuclei, dorsal endopiriform nucleus, piriform cortex, posterolateral cortical amygdaloid nucleus, central nuclei and lateral nuclei. There also was minimal damage to the posteromedial cortical amygdaloid nucleus, intraamygdaloid division of the stria terminalis bed nucleus, caudate putamen and globus pallidus. Except for the piriform cortex which sustained 50% bilateral damage, these additional areas only had unilateral lesions.

The NMDA animals sustained damage centered in the basolateral region but the extent of anterior-posterior damage varied from 20-100% with an average of 50% (see Figure 2). Extensive damage to the basomedial nuclei, dorsal endopiriform nucleus, and lateral nuclei and minor damage to the piriform cortex, posterolateral cortical amygdaloid nucleus, central

nuclei, posteromedial cortical amygdaloid nucleus, intraamygdaloid division of the stria terminalis bed nucleus, caudate putamen and globus pallidus also was sustained. It is important to note that no animals were observed to have seizures (a possible source of damage) following NMDA infusion. Photographs of representative sections from CONT, SHAM, ELEC and NMDA animals are shown in Figure 3.

Behavior. The four groups of Acquisition-Before males did not differ in the amount of sucrose consumed on the day of acquisition (see Figure 4). During the retention test, the ELEC males drank more sucrose than any of the other groups ($F[3,26]=9.39$, $p=.0002$; Newman-Keuls, $p<.05$).

The four groups of Acquisition-After males did not differ in the amount of sucrose consumed on the day of acquisition. During the retention test, the ELEC males drank more sucrose than any of the other groups ($F[3,29]=11.60$, $p=.0001$; Newman-Keuls, $p<.05$).

Conclusion

Like Dunn and Everitt (1988) we found that acquisition of a conditioned taste aversion was attenuated in males with electrolytic but not neurotoxic lesions of the basolateral amygdala. These results suggest that the basolateral amygdala does not play a role in acquisition of conditioned taste aversions.

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APPENDIX E

Sengstake, C. B. and Chambers, K. C. Sensitivity of male, female, and androgenized female rats to testosterone during extinction of a conditioned taste aversion. Behavioral Neuroscience, in press.

Sensitivity of Male, Female, and Androgenized Female
Rats to Testosterone during Extinction
of a Conditioned Taste Aversion

Cord B. Sengstake

Portland State University

and

Kathleen C. Chambers

University of Southern California

Running Head: Testosterone Sensitivity

Abstract

The hypothesis that males and females differ in the amount of testosterone required to prolong extinction of a conditioned taste aversion was tested. Gonadectomized males and females were implanted with empty capsules or 30, 60, or 120 mm long testosterone-filled capsules. Blood samples were taken before conditioning and after extinction. Both males and females exhibited slow extinction rates when given a 120 mm capsule but when both were implanted with either a 30 mm or a 60 mm capsule, only males showed the slow extinction rate. The dimorphic sensitivity could not be attributed to differences in plasma testosterone levels since the levels for males and females with either 30 mm or 60 mm capsules were not different. In experiment 2 the hypothesis that the presence of testosterone in the male during the perinatal period results in a greater sensitivity to testosterone in adulthood was tested. Females exposed to testosterone propionate during the perinatal period showed prolonged extinction when given a 30 mm testosterone-filled capsule as an adult whereas unexposed females did not. These results support the hypothesis that the amount of testosterone required to activate the prolonged extinction is dependent on the perinatal presence of testosterone.

Sensitivity of Male, Female, and Androgenized Female
Rats to Testosterone during Extinction
of a Conditioned Taste Aversion

The influences of gonadal hormones on both reproductive and nonreproductive sexually dimorphic behaviors have been placed within the framework of organizational (perinatal) and activational (adult) effects (Goy & Goldfoot, 1973). Under this formulation, the various dimorphic behaviors were classified according to the relative importance of the two stages of hormonal action. Many dimorphic behaviors were seen as clearly dependent on both organizational effects and activational effects. In this case, adult gonadectomy would be expected to block the expression of the behavior and adult treatment of the opposite sex with the appropriate hormone should result in a diminished response at best. For example, adult castration of male mice results in a reduction in same sex aggression and exogenous testosterone (T) treatment results in a return to pre-castration levels. Injections of T in adult female mice, however, do not result in levels of aggression equal to those of normal males (Tollman & King, 1956; Simon & Whalen, 1987).

Other behaviors were seen as primarily dependent on the hormonal levels during a sensitive part of the perinatal period, i.e., organizational effects. In this case, the presence or absence of gonadal hormones would be expected to

determine whether the organism develops the neural system required for that behavior to be exhibited as an adult. Gonadectomy or exogenous hormone administration in the adult animal should have only minimal effects on the behavior. An example of an organizationally determined behavior is open-field behavior in the rat (Bengelloun, Nelson, Zent, & Beatty, 1976). Females are more active than males in open field tests. Adult gonadectomies in male and female rats have little effect on this behavior but neonatal castration of the male results in female levels of activity when tested as an adult; injections of testosterone propionate (TP) in the neonatal female results in male levels as an adult.

Activationally determined behaviors were thought to be dependent upon the presence of the appropriate gonadal hormone(s) in the adult regardless of the hormonal conditions during the perinatal period. Adult gonadectomy would result in a decrease in the behavior while injections of the appropriate hormone would result in the full expression of the behavior in both sexes. An example of an activationally determined behavior is the rate of extinction of a conditioned taste aversion in rats (Chambers, 1976). Castration of the adult male results in female-like behavior (i.e., a rapid rate of extinction); exogenous administration of TP in the adult rat of either gender results in male-like behavior (i.e., a slow rate of extinction).

A major difference, then, between behaviors that are activational and behaviors that are both activational and organizational is the relative sensitivity of the target tissues in the opposite sex to the activating hormone. In the case of behaviors that are both activational and organizational, the sensitivity of the adult to the hormone is clearly dimorphic; the issue is less clear for behaviors that are activationally determined.

In our studies of the extinction of a conditioned taste aversion (Sengstake, Chambers, & Thrower, 1978; Chambers & Sengstake, 1979), for example, we have not observed a sex difference in the responsiveness to T; the males and the females given daily injections of TP have not differed in their rate of extinction. However, only relatively high doses of TP were used; 1 mg per rat per day. Differences in the responsiveness of males and females to T may well have been obscured by these high doses. Thus, the following studies were designed to determine whether males and females differ in their sensitivity to T and whether perinatal hormones play a role in conditioned taste aversions.

General Method

Husbandry

The subjects were Sprague-Dawley-derived rats. After weaning, they were housed in same-sex colony cages until one week before conditioning. During this period and continuing

throughout the experiment, they were maintained on ad libitum food and water and were housed in a temperature-controlled (22° - 23° C) vivarium with a 12h:12h light/dark cycle (lights on at 0230).

Implants

T-filled implants were made by a method derived from Legan, Coon, and Karsch (1975). Silastic tubing (0.062 in. ID, 0.125 in. OD) was cut into lengths 3 mm longer than the desired final length. Crystalline T was pushed into each tube with a 17-gauge stainless steel rod. Because the rod fit snugly into the tube, the tube was filled with no air spaces. When the appropriate length of the column had been obtained, the ends were sealed with a small amount of type A Silastic adhesive, and allowed to cure overnight. Prior to surgical placement, the tubes were incubated for 72 hr at 37° C in 0.01 M phosphate-buffered (7.4 pH) normal saline; the buffered saline solution was replaced twice a day during this incubation.

Conditioned Taste Aversion

The conditioned taste aversion was induced as follows. At the beginning of the dark phase of the light/dark cycle, the water bottle of each rat was replaced with a chilled sucrose solution (10% sucrose in water, wt/vol) in a graduated cylinder for 2 hr. The amount consumed was recorded, the sucrose solution was replaced with the regular

water bottle, and the animal received an injection of a 0.3 M LiCl solution (20 ml/kg of body weight, intraperitoneally). Two days after the acquisition of the aversion, daily extinction trials began. The extinction trials were conducted in the same manner as the acquisition session except that no LiCl was injected.

Radioimmunoassay

T was separated from the plasma (Experiment 1) or serum (Experiment 2) through extraction and chromatographic purification on LH-20 Sephadex columns and quantified by radioimmunoassay (Resko, Malley, Begley, & Hess, 1973; Resko, Ellinwood, Pasztor, & Buhl, 1980). Percentage of recovery, water blank values, and intraassay and interassay coefficients of variations were determined and quantities calculated from standard curves were corrected for procedure losses and blank values.

Experiment 1

This experiment was designed to determine whether males and females differ in the amount of T required to prolong extinction of a conditioned taste aversion and to determine the circulating T levels required in both males and females for a slow extinction.

Method

Part A: The subjects were 30 male and 30 female rats. They were randomly assigned to one of five conditions (six

males (M) and six females (F) per condition): gonadectomized with one 30 mm (30), one 60 mm (60), or two 60 mm (120) T-filled Silastic capsule implants; gonadectomized with one 30 mm empty capsule implant (0); or sham-gonadectomized with one 30 mm empty capsule implant (S). When the rats were 90-120 days old, they were gonadectomized or underwent sham operations with sodium pentobarbital anesthesia (36-48 mg/kg of body weight, intraperitoneally). Fourteen days later each animal received the subcutaneous Silastic implant under ether anesthesia.

Two weeks after implantation, each rat was moved to an individual cage that was to serve both as the testing and the home cage for the duration of the experiment. Beginning the next day and continuing daily throughout the experiment, each rat was weighed at the end of the light portion of the light:dark cycle. Also for the first seven days the water bottle of each animal was replaced with chilled tap water in a graduated cylinder for the first 2 hr of the dark phase. After 1 week of this preconditioning, the conditioned taste aversion was induced. Extinction continued either until the rat regained the consumption level shown on the acquisition day or until there had been 41 extinction trials (whichever occurred first). The extinction score for each animal was the number of trials to extinction, or 42 if no extinction occurred.

Part B: The subjects were 30 male (M) and 30 female (F) rats. They were randomly assigned to one of 5 conditions (8 males and 8 females per condition): gonadectomized with one 30 mm (30), one 60 mm (60), or two 60 mm (120) T-filled capsule implants; gonadectomized with one 30 mm empty capsule implant (0); or sham-gonadectomized with 30 mm empty implant (S).

The procedure was identical to that of Part A with the following exceptions. Blood samples were taken from each animal 1 and 2 weeks after the implantation. The day after the second sampling, each rat was moved to an individual cage. Two additional blood samples were taken from each animal at the end of the extinction trials, 73 and 80 days after the initial implantation. The blood samples were taken from the tail veins of etherized animals with heparinized syringes and were stored on ice for no more than 30 min before being centrifuged (2,000 rpm/700 x g for 20 min at 4° C). The plasma was stored at -18° C until assayed for T. The mean (\pm SE) percentage of recovery and blank value, and the intraassay and interassay coefficients of variation were: 73.69(\pm 2.40)%, 9.90(\pm 4.64) pg/ml, 12.61% and 6.77% respectively.

Results

The data on two (one 60F and one 0F) animals that died during the experiment in Part B were excluded from all

calculations and analyses. The mean levels of T in blood samples 1 and 2 (preconditioning trials) and the mean level in samples 3 and 4 (postextinction trials) were computed for each animal. Both the days to extinction and the mean levels of T data were analyzed using a one factor ANOVA followed by selected linear contrasts.

For both Part A and B, the extinction rates of the 10 groups differed (Part A: $F(9,50) = 6.37$, $p < .001$; Part B: $F(9,48) = 3.79$, $p = .001$; see Figure 1). Neither the extinction rates of the OM and the OF (Part A: $F(1,50) = 0.01$; Part B: $F(1,48) = 0.76$), nor the 120M and 120F (Part A: $F(1,50) = 0.31$; Part B: $F(1,48) = 3.09$) differed but the extinction rates of the SM and SF (Part A: $F(1,50) = 8.27$, $p = .006$; Part B: $F(1,48) = 4.39$, $p = .04$) and the extinction rates of the 30M and the 30F (Part A: $F(1,50) = 7.18$, $p = .01$; Part B: $F(1,48) = 5.42$, $p = .02$) were different. The extinction rates of the 60M and the 60F differed in Part A ($F(1,50) = 8.59$, $p = .005$) but not in Part B ($F(1,48) = 0.90$). When the mean days to extinction were calculated for the combined data from Part A and Part B for the 60M and the 60F, however, the two genders were different ($F(1,108) = 6.33$, $p = .013$).

Insert Figure 1 about here.

The T levels of the 10 groups differed both before conditioning and after extinction ($F(9,48) = 24.29$ and 22.31 , respectively, with $p < .001$ in both cases; see Figure 2). The SM had higher T levels than the SF both before and after conditioning ($F(1,48) = 13.14$, $p = .001$, and 4.47 , $p = .04$, respectively). The T levels did not differ significantly between the sexes either before conditioning or after extinction in the following groups: the OM and OF ($F(1,48) = 0.64$ and 0.02 respectively), the 30M and 30F ($F(1,48) = 0.08$ and 0.61 respectively) or the 60M and 60F ($F(1,48) = 0.002$ and 0.96 respectively). Although the T levels of the 120M and 120F did not differ before conditioning ($F(1,48) = 1.57$), they were significantly higher in the 120F than in the 120M after extinction ($F(1,48) = 19.47$, $p < .001$).

Insert Figure 2 about here.

Experiment 2

In experiment 1, when males and females were given either 30 or 60 nm of T, the extinction rate of the males was slower than that of the females. This sex difference cannot be accounted for by different levels of circulating T since neither the males and females given 30 nm of T nor the males and females given 60 nm of T differed significantly in plasma T levels.

Although several other hypotheses can be offered to account for this dimorphic sensitivity to T, a very likely explanation is the presence of T in males during differentiation of the central nervous system. In support of this hypothesis is the study by Babine and Smotherman (1984) in which they found that female rats presumably exposed to T prenatally from neighboring male siblings show a prolonged extinction as adults when given low-dose injections of T, whereas females without neighboring male siblings (and less prenatal T) do not. The following experiment was designed to determine if sensitivity to T, as a factor in determining extinction rate, is established during the perinatal period. Under this hypothesis we predicted that the amount of T required to prolong extinction is less in females that have been exposed to exogenous T during the perinatal period compared to females not so exposed.

Method

Four females were injected daily with 2 mg of TP (dissolved in 0.1 ml of sesame oil) and another four were injected daily with 0.1 ml of the oil on days 16 through 20 of pregnancy. Twenty-four and 72 hr after delivery, the pups of the TP-injected females were injected with 1 mg of TP (dissolved in 0.02 ml of sesame oil) and the pups of the oil-injected females were injected with 0.02 ml of the oil. The pups were weaned when 22 days old.

When the offspring were 80 days old, all of the TP-treated females (n = 10) were selected and 16 oil-treated females and 8 oil-treated males were randomly selected to be subjects in this experiment. All of the subjects were gonadectomized and implanted with silastic capsules while under ether anesthesia. All of the males (OTM) and half of the TP-treated (TTF) and oil-treated (OTF) females were implanted with 30 mm T-filled capsules. The other half of the TP-treated (TOF) and oil-treated (OOF) females were implanted with empty 30 mm capsules. One week later, the rats were placed in single cages and 1 week after that the conditioned taste aversion was induced. Extinction continued either until the rat regained the consumption level shown on the acquisition day or until 29 extinction trials had been given (whichever occurred first). The extinction score for each animal was the number of trials to extinction, or 30 if no extinction occurred.

Two days after the last extinction trial, all of the animals were bled. Blood samples were taken from a tail vein under vacuum by the method of Nerenberg and Zedler (1975). The animals were unanesthetized. The blood was allowed to clot at 40 C. It was centrifuged at 5000 rpm (4000 x g) at 40 C for 30 min. The serum was removed and stored in 1 ml aliquots at -200 C until assayed for T.

Results

The extinction and T data were analyzed using a one factor ANOVA followed by selected linear contrasts. The five groups did not all have the same extinction rate ($F(4,29) = 9.09$, $p < .001$; see Figure 3). The extinction rates of the OOF and the TOF groups did not differ ($F(1,29) = 1.13$), and they did not differ from the OTF animals ($F(1,29) = 2.33$). The rates of the TTF and OTM animals also were not different ($F(1,29) = 0.79$). However, the extinction rates for the TTF and the OTM animals were significantly slower than those of the OOF, TOF, and OTF animals ($F(1,29) = 31.39$, $p < .001$).

Insert Figure 3 about here.

The five groups also did not all have the same serum level of T ($F(4,29) = 38.17$, $p < .001$; see Figure 4). The T levels of the OTF and the OTM animals did not differ ($F(1,29) = 2.12$) nor did they differ from the TTF animals ($F(1,29) = 0.84$). The T levels of the OOF and the TOF animals were not different ($F(1,29) = 0.12$). As expected, the T levels of the rats with T-filled capsules (OTF, OTM, and TTF) were significantly higher than those with empty capsules (OOF and TOF) ($F(1,29) = 143.36$, $p < .001$).

Insert Figure 4 about here.

General Discussion

The amount of T required to produce a slow extinction rate is altered by the presence of T during the perinatal period. Adult gonadectomized rats that had no exogenous T present during the perinatal period (normal females) exhibited a fast rate of extinction when given a 30 mm T-filled capsule whereas adult gonadectomized rats that had T present during the perinatal period (males and androgenized females) showed a slow extinction rate when given a 30 mm T-filled capsule. Thus, the presence of T during the perinatal period results in an increased sensitivity of the neural substrate to T for this behavior in the adult animal.

The presence of T during the perinatal period also has been found to influence sensitivity to T in adulthood for other behaviors dependent on the activational effects of gonadal hormones. Gonadectomized male mice require less T to activate intermale aggression than neonatally castrated males or gonadectomized females (Bartley & Goldman, 1977a, b; Simon & Whalen, 1987). However, although a higher dose of T activates aggression in adult females, it does not produce levels equal to those of normal males (Tolman & King, 1956; Simon & Whalen, 1987). T prolongs extinction of a conditioned taste aversion in normal adult females but whether there is a dose of T that produces the same extinction rate in males and females remains unclear.

Although we failed to find a behavioral difference between males and females given 120 mm of T, this may have been due to our limiting the number of extinction trials to 41.

The results from Experiment 1 suggest that 60 mm of T is a near threshold dose of T for normal females as a group. A reexamination of the extinction data in terms of the percentage of animals with extinction scores of 20 or greater revealed that 83-100% of the animals from those groups with a slow extinction (sham males, gonadectomized males with 30, 60, or 120 mm capsules, and gonadectomized females with 120 mm capsules) and 8 - 23% of the animals from those groups with a fast extinction (sham females, gonadectomized males and females with no T and gonadectomized females with 30 mm capsules) extinguished after 20 or more days. The percentage of females with 60 mm capsules whose extinction was 20 days or longer (45%) fell in between the range of values of those groups with a slow and those with a fast extinction. It may be that at least part of the individual differences found in female sensitivity to 60 mm of T is due to their in utero environment. Babine and Smotherman (1984) have found that female rats with males on both sides in utero showed prolonged extinction as adults when given low-dose injections of T whereas female rats with females on both sides in utero did not.

The T levels of females with 60 mm capsules were not different from those of intact males ($t(9) = 0.79$). The range of T values found in intact males was 1.53 - 4.18 ng/ml and the range for those females with 60 mm capsules that extinguished after 20 or more days was 2.77 - 4.44 ng/ml. This indicates that extinction can be prolonged in at least some females by doses of T that produce T values that are within the range of values found in intact males.

In his review, Beatty (1979) stated that no sexually dimorphic behavior has been shown to be without some early organizational influence. The sexual dimorphism in the extinction of a conditioned taste aversion is no exception. The variation between behaviors seems to be in the relative dominance of the organizational and activational effects. Those behaviors that seem to be primarily organizational result from one gender being much more sensitive to the hormone than the other gender. To the extent that the behavior seems to have little organizational influence, the two genders are much more equal in their sensitivity to the hormone. Thus, the amount of disparity between genders in sensitivity to the activational effects of gonadal hormones for the different sexually dimorphic behaviors could be seen as a continuum; at one end we would find small differences in sensitivity, such as is the case for the extinction of a conditioned taste aversion, and at the other extreme we would

find large differences in sensitivity, such as is the case for aggression in mice and open-field behavior in rats.

From the results of Experiment 2, it is clear that the sexual dimorphic rate of extinction is dependent on T action during adulthood. Those adult gonadectomized rats that had T present during the perinatal period (males and androgenized females) did not exhibit a slow rate of extinction unless T was present during testing. Animals that had little or no T during the developmental period (females) displayed the slow rate of extinction if given sufficient T in adulthood. Thus, although the presence of T during the perinatal period alters subsequent sensitivity to T, it is the presence of sufficient T during adulthood, and not the presence of this hormone during the perinatal period that is critical for the expression of a slow extinction rate.

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Author Notes

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Requests for reprints should be addressed to Cord B. Sengstake, Department of Psychology, Portland State University, P. O. Box 751, Portland, OR 97201.

Figure Captions

Figure 1. Mean (\pm SE) days to extinguish a conditioned taste aversion in sham females (SF), sham males (SM), gonadectomized females (light shading) and gonadectomized males (dark shading) implanted with empty silastic capsules (0) or 30, 60, or 120 mm testosterone-filled capsules for Experiments 1A and 1B. *Significantly different than the males in the same treatment condition, $p < .05$.

Figure 2. Mean (\pm SE) serum testosterone levels (ng/ml) in sham females (SF), sham males (SM), and gonadectomized females (light shading) and gonadectomized males (dark shading) implanted with empty silastic capsules (0) or 30, 60, or 120 mm testosterone-filled capsules before acquisition of a conditioned taste aversion (Pre-Acquisition) and after extinction (Post-Extinction). *Significantly different than males in the same treatment condition, $p < .05$.

Figure 3. Mean (\pm SE) days to extinguish a conditioned taste aversion in males treated with oil perinatally and testosterone (T) during testing as an adult (OTM), females treated with oil perinatally and no T during testing (OOF), females treated with oil perinatally and T during testing (OTF), females treated with testosterone propionate (TP) perinatally and no T during testing (TOF), and females

treated with TP perinatally and T during testing (TTF).

*Significantly different than OTM, $p < .05$.

Figure 4. Mean (\pm SE) serum testosterone levels (ng/ml) in males treated with oil perinatally and testosterone (T) during testing as an adult (OTM), females treated with oil perinatally and no T during testing (OOF), females treated with oil perinatally and T during testing (OTF), females treated with testosterone propionate (TP) perinatally and no T during testing (TOF), and females treated with TP perinatally and T during testing (TTF). *Significantly different than OTM, $p < .05$.

Fig 1

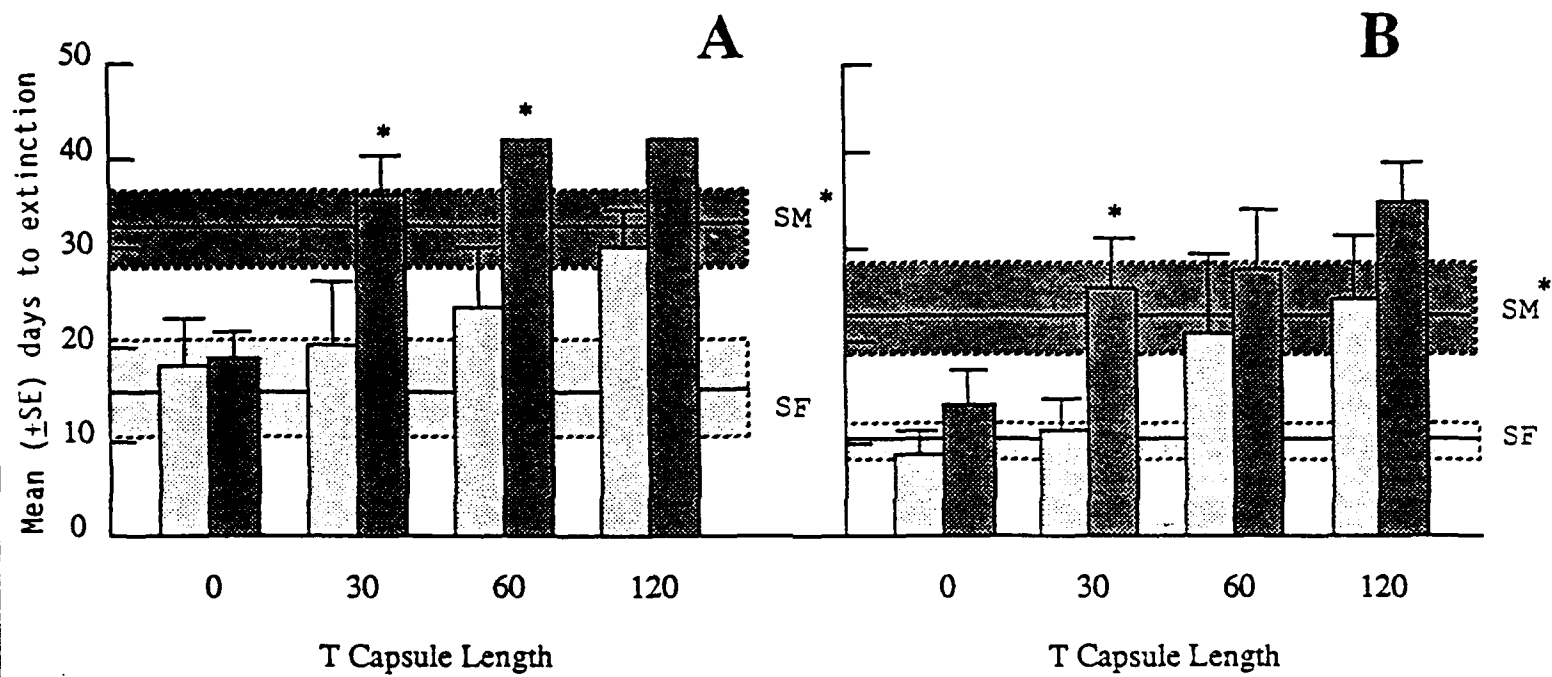


Fig 2

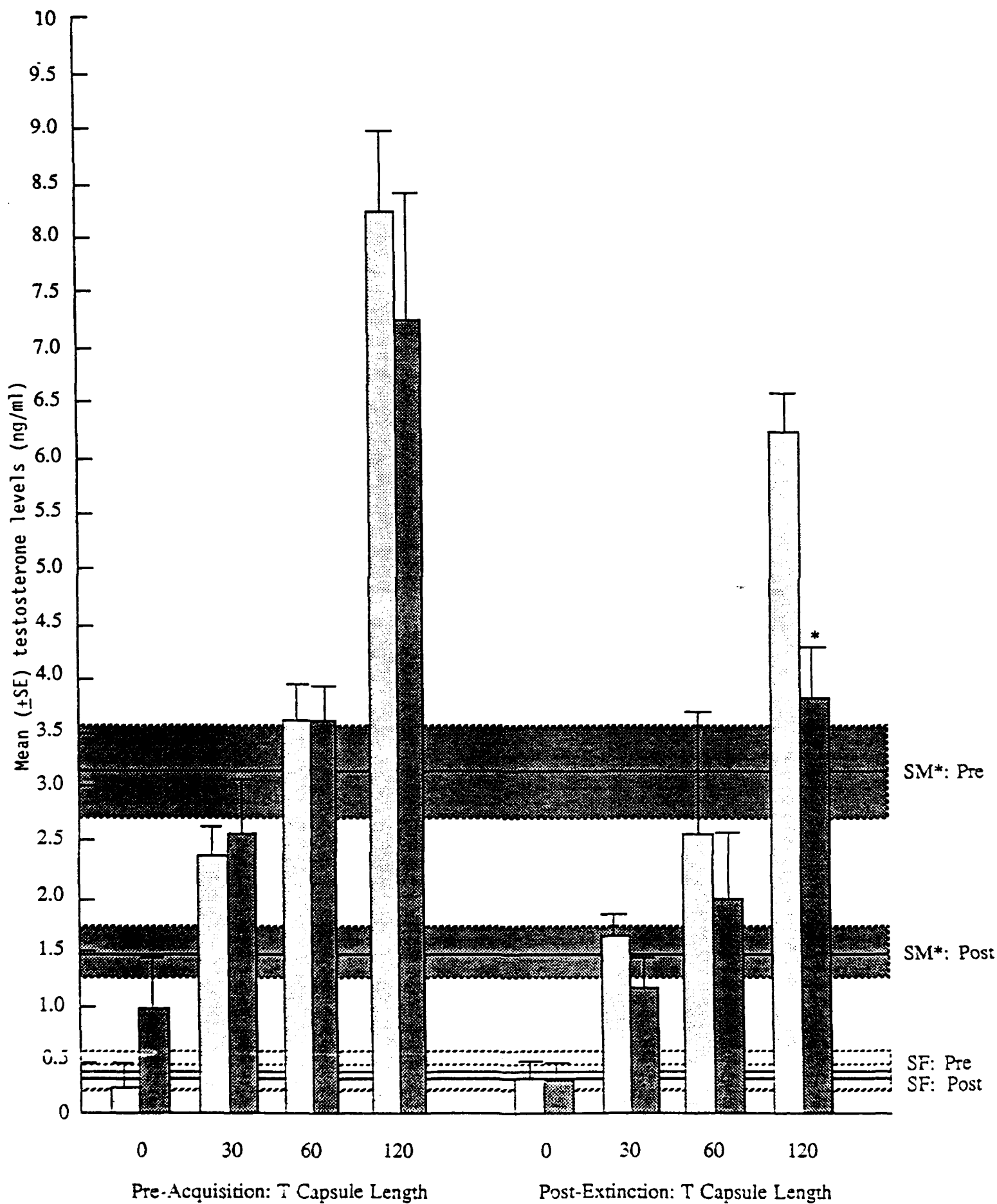
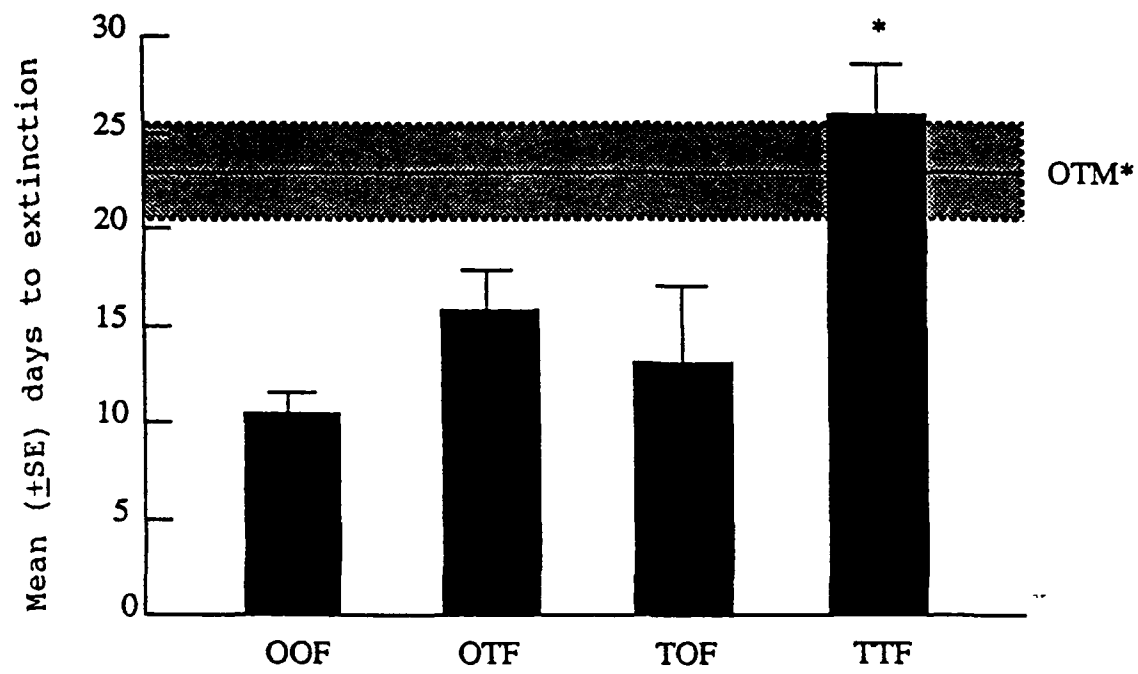
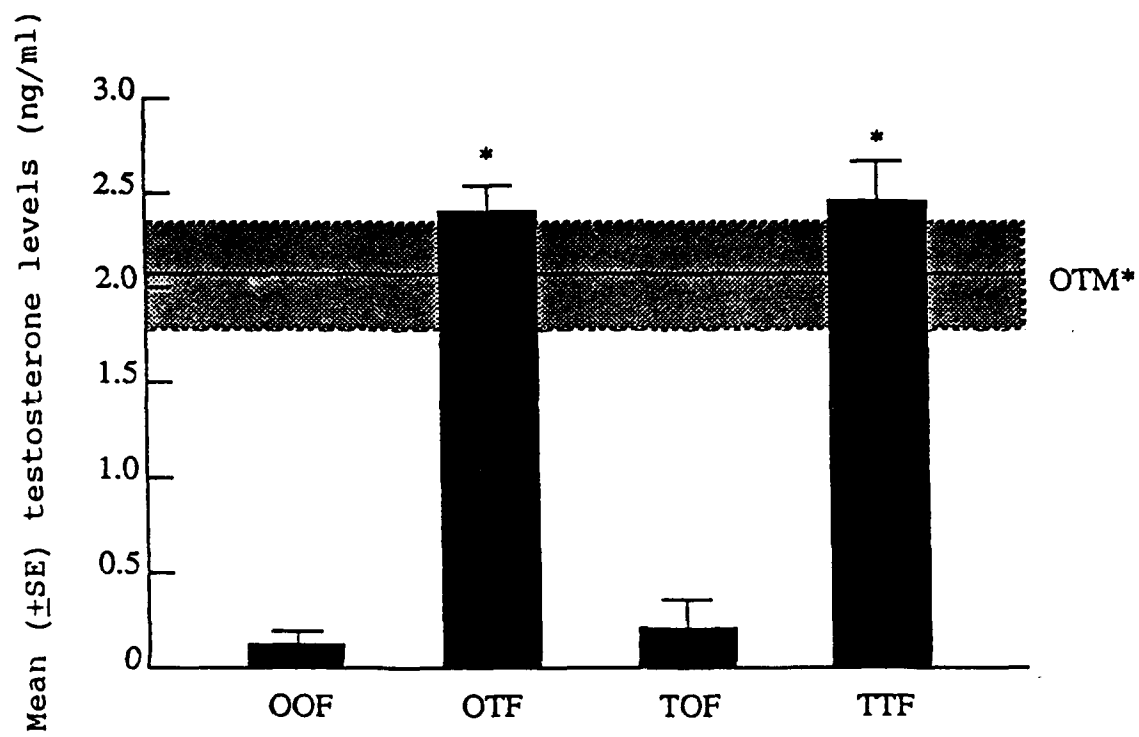


Fig 3





APPENDIX F

Effects of gonadectomy on extinction
of a conditioned food aversion

David L. Yuan, Charles Hung and Kathleen C. Chambers

Poster presented at the Western Psychological Association meeting
Los Angeles, CA
April 1990

EFFECTS OF GONADECTOMY ON EXTINCTION OF A CONDITIONED FOOD AVERSION.

D.L. Yuan, C. Hung, and K.C. Chambers

Department of Psychology, USC, Los Angeles, CA 90089

INTRODUCTION

Males extinguish a conditioned food aversion more slowly than females. This sexual dimorphism is due to the higher levels of testosterone found in males than in females. Gonadectomy increases extinction rates in males but has no effect in females. Testosterone treatment prolongs extinction in both gonadectomized males and gonadectomized females.

We have observed strain differences in the degree to which gonadectomy accelerates extinction in males. In Sprague-Dawley rats, gonadectomy increases the extinction rate of males to the rate exhibited by females. In Fischer 344 rats, however, gonadectomized males show slower extinction rates than females. One possible explanation for this strain difference is that the effect of gonadectomy is expressed more slowly in Fischer 344 rats than in Sprague-Dawley. In previous studies, a conditioned food aversion was induced 1 week after gonadectomy. It has been shown in studies of reproductive behavior that the decrease in male sexual behavior after gonadectomy can take weeks to be expressed. Thus the following experiment was designed to

determine whether the extinction rates of Fischer 344 rats vary with the length of time after gonadectomy.

METHOD

Twenty male and 20 female Fischer 344 rats were randomly assigned to one of two groups: 5 week delay or 1 week delay. All rats were 90 days old at the start of the study.

A conditioned food aversion was induced 5 weeks after gonadectomy in half of the males and females and 1 week after gonadectomy in the other half. On the day of acquisition, all of the rats were given access to a 9% sucrose solution for 1 hour at the beginning of the dark portion of the light/dark cycle. Immediately after this access period, all of the rats were injected with a 0.15M LiCl solution (10 ml/kg of body weight). Daily extinction trials were initiated 2 days later and were continued until criterion for extinction (100% of acquisition day consumption) was reached.

RESULTS

On the day of acquisition the four groups of rats did not differ in the amount of sucrose consumed.

The four groups of males differed significantly in the rates of extinction exhibited ($F[3,36] = 11.38, p < .0001$).

* The 1-week males exhibited significantly slower extinction rates than the 5-week males (Newman-Keuls Test, $p < .05$). The extinction rates of the two groups of females did not differ.

** Both groups of males, however, still exhibited slower extinction rates than both groups of females (Newman-Keuls Test, $p < .05$).

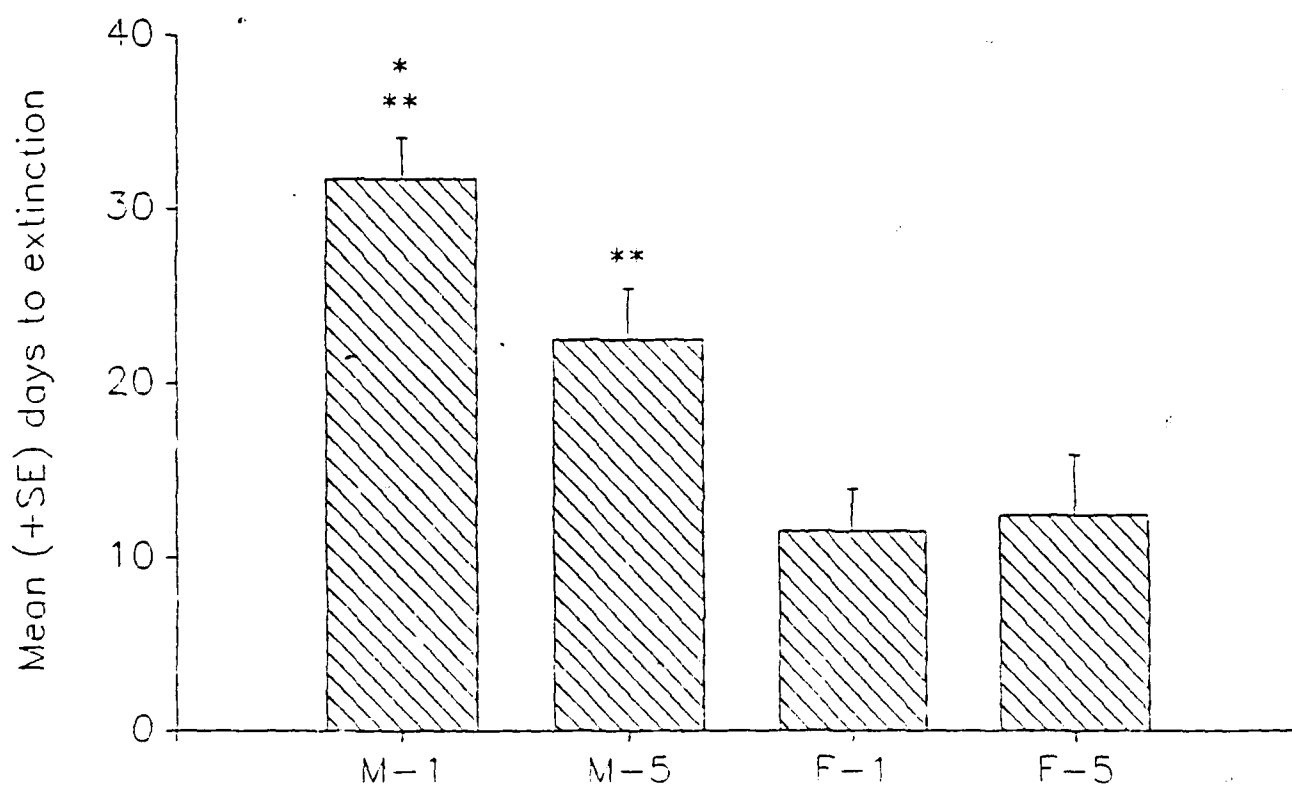
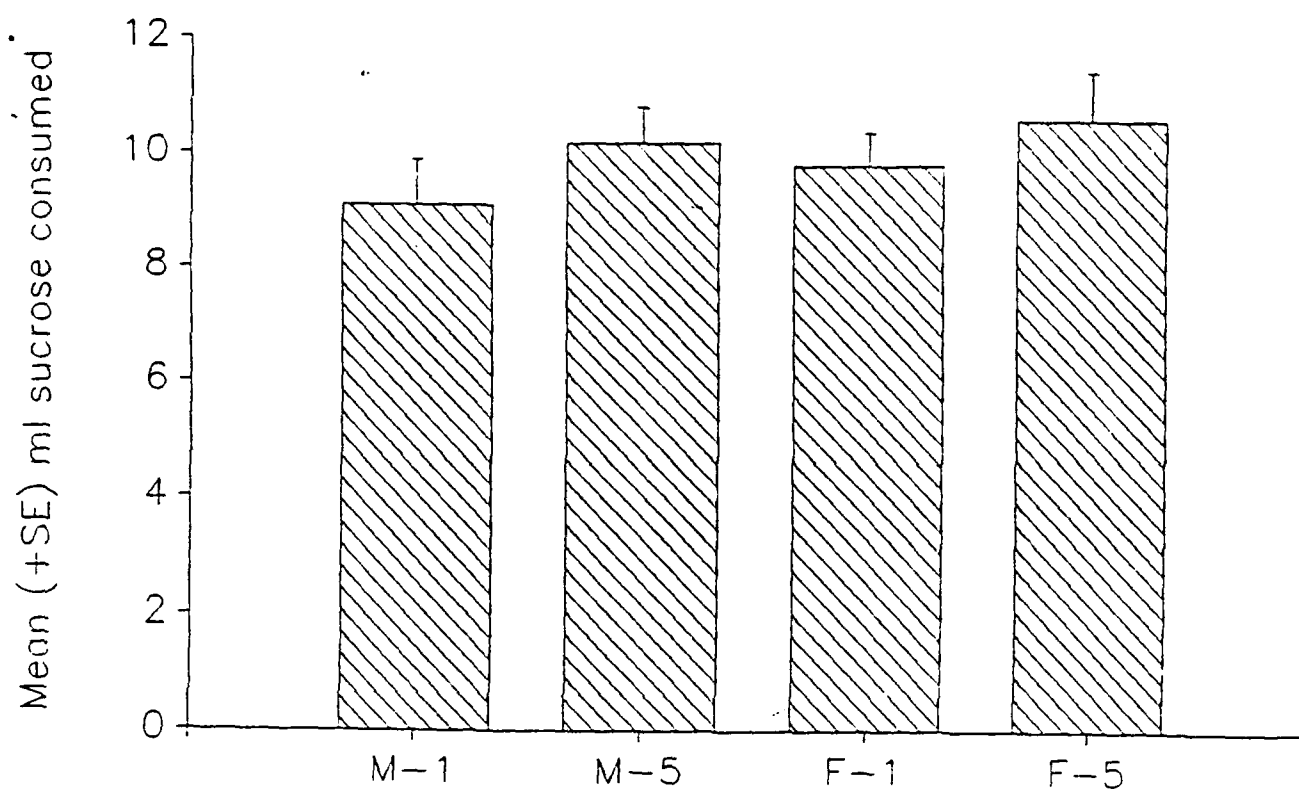
CONCLUSION

These results suggest that differences in the effects of gonadectomy on extinction rates in Sprague-Dawley and Fischer 344 rats may be accounted for, at least in part, by differences in the length of time it takes the full effects of gonadectomy to be expressed.

The failure to completely eliminate the sexual dimorphism after gonadectomy in Fischer 344 rats may be due to an insufficient time delay between gonadectomy and behavioral testing.

It also is possible that hormonal influences during perinatal development are more important in Fischer 344 than in Sprague-Dawley rats.

Supported by grants HD 20970 and ONR N00014-89-J-1296.



M-1: Male 1-wk
F-1: Female 1-wk

M-5: Male 5-wks
F-5: Female 5-wks

Appendix G

To be presented at the American Psychological Association meeting, Boston, 1990.

ABSTRACT

CONDITIONED FOOD AVERSION IN YOUNG AND OLD MALE RATS.

David L. Yuan, Charles Hung, and Kathleen C. Chambers. Department of Psychology, USC, Los Angeles, CA 90089-1061

Previous studies have shown that old male rats exhibit greater resistance to extinction of a conditioned food aversion than young males. This study was designed to determine the nature of the age-related difference 1) by varying the time interval between acquisition and extinction and 2) by varying the dosages of the illness-inducing agent, LiCl. The results indicate that the retention and extinction processes of old male rats are more resistant to the effects of the passage of time. However, young rats are more sensitive to a lower dosage of LiCl. Supported by grants NIH-HD20970 and ONR-N00014-89-J-1296.

SUMMARY

CONDITIONED FOOD AVERSION IN YOUNG AND OLD MALE RATS. David L. Yuan, Charles Hung, and Kathleen C. Chambers. Department of Psychology, USC, Los Angeles, CA 90089-1061

Previous studies have shown that old male rats exhibit greater resistance to extinction of a conditioned food aversion than young males. This study was designed to determine the nature of the age-related difference 1) by varying the time interval between acquisition and extinction and 2) by examining the difference in old and young male rats in their sensitivity to the illness-inducing agent, LiCl, and therefore their ability to acquire a conditioned food aversion.

In the first experiment, twenty young (3.3-4.3 months) and 26 old (18 months) Fischer male rats were randomly assigned to four groups: young rats with 1 day delay between acquisition and extinction (young 1-day-delay group), young rats with 1 month delay between acquisition and extinction (young 1-month-delay group), old rats with 1 day delay between acquisition and extinction (old 1-day-delay group), and old rats with 1 month delay between acquisition and extinction (old 1-month-delay group). The rats were housed two per cage and were kept on a 12:12 hr light/dark cycle. On the day of acquisition (Day 1), the rats were given access to a chilled 10% sucrose solution (wt/vol in water) at the beginning of the dark phase of the cycle. One hour later, the conditioned food aversion was induced

by injection of LiCl (0.15 M, 10 ml/kg). Acquisition for the 1-day-delay and 1-month-delay groups was given at different times. For all groups the first extinction trail was given at the same time. Daily extinction trials were continued until criterion for extinction (100% of first day consumption) was reached.

The extinction rates of the young 1-month-delay group were significantly faster than those of the young 1-day-delay group and the two old groups ($F(3,40)=4.80$, $p=.005$). The extinction rates of the two groups of old males and the young 1-day-delay males did not differ statistically. Clearly, the retention and extinction processes of old male rats are less subject to the effects of the passage of time than are those of young males.

The extinction process is affected by the strength of acquisition. In the aging studies, differences in the retention or extinction processes could be accounted for by differences in the initial level of acquisition in old and young animals. In the previous studies, old and young males were given the same dosage (per body weight) of illness-inducing agent. It may be that old males have an increased sensitivity to the agent. We tested this hypothesis in the second experiment by pairing several different dosages of LiCl repeatedly with a sucrose solution. We then compared the proportion of old and young animals that acquired an aversion at each dosage level.

Forty young male rats (3 months) and 38 old male rats (16 months) were randomly assigned to 1 of 4 groups: 1.00 (Group 1), 0.625 (Group 2), 0.250 (Group 3), and 0.125 (Group 4) mg/kg of body weight of LiCl. On the first acquisition day, immediately after the one hr sucrose solution presentation, rats received intraperitoneal injections of LiCl according to their group assignment. On Day 2 the rats were given access only to the chilled tap water for 1 hr. For the next 22 days, there was an alternation of acquisition and recovery days with the same experimental procedure used on days 1 and 2, respectively. The experiment was terminated on Day 24. Once a rat reached a complete aversion (consumption of 2 ml or less of the sucrose solution during any 1-hr test period) it was no longer given LiCl injections.

The results indicate that all old and young rats in Group 1 acquired an aversion. Eight out of ten young rats in Group 2 acquired an aversion but only 2 old rats did. There were 4 and 1 young rats in Groups 3 and 4, respectively, that acquired an aversion but none of the old rats in these group did. The difference in the proportion of animals in the four groups of young and old males that acquired an aversion was significant ($\chi^2(3)=57.06$, $p<0.01$). Young male rats tend to be more sensitive to a lower dosage of LiCl. These results do not support the hypothesis that the slower extinction observed in old male rats can be accounted by the increased sensitivity to LiCl in the old rats.

Supported by grants NIH HD20970 and ONR N00014-89-J-1296.

APPENDIX H

Failure to observe age-related differences in extinction
of a conditioned food aversion when low doses of LiCl are used

David L. Yuan, Charles Hung and Kathleen C. Chambers

Poster to be presented at the Gerontological Society meeting
Boston, MA
November 1990

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FAILURE TO OBSERVE AGE-RELATED DIFFERENCES IN
EXTINCTION OF A CONDITIONED FOOD AVERSION
WHEN LOW DOSES OF LiCl ARE USED. D.L. Yuan,
C. Hung, K.C. Chambers, Department of
Psychology, USC, Los Angeles, CA 90089-1061

Old male rats exhibit slower rates of extinction of a conditioned food aversion than young males when a single high dose of toxin is administered. This study was designed to determine if this age-related difference also is observed when low doses of toxin are used. Forty young and 38 old male rats were assigned randomly to one of four groups: 1.0, 0.625, 0.25, or 0.125mg/kg of body weight of LiCl. All males were given access to a 9% sucrose solution for 1 hr. Then they were immediately given intraperitoneal injections of their assigned dose of LiCl. This procedure was repeated every other day until each male acquired an aversion (consumed 1.5 cc or less). Extinction tests then were initiated and were continued every other day until the male regained the consumption level exhibited on the first day of acquisition. For those old and young males that acquired an aversion at a given dose, no differences in acquisition rate or extinction rate were found. These results suggest that old and young males differ in how they process information about LiCl when high doses are given but not when low doses are given. Supported by grants NIH-ED20970 & ONR-N00014-89-J-1296

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APPENDIX I

Effects of fluid deprivation on testosterone sensitivity and
extinction of a conditioned taste aversion

Elizabeth A. Brownson, Cord B. Sengstake and Kathleen C. Chambers

Poster to be presented at the Society for Neuroscience meeting
St. Louis, MO
October 1990

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EFFECTS OF FLUID DEPRIVATION ON TESTOSTERONE SENSITIVITY
AND EXTINCTION OF A CONDITIONED TASTE AVERSION. E.A.
Brownson, C.B. Sengstake* and K.C. Chambers. Depts. of
Neurobiology and Psychology, Univ. of So. Cal., Los
Angeles, CA 90089 and Dept. of Psychology, Portland State
Univ., Portland, OR 97207.

Fluid deprivation reduces behavioral sensitivity to
testosterone (T). The amount of T required to prolong
extinction of a conditioned taste aversion (CTA) is
greater in fluid deprived than nondeprived male rats. In
the first experiment, we observed that the minimum dose of
T required to prolong extinction in fluid deprived
Sprague-Dawley (SD) male rats is not sufficient in Fischer
344 (F344) males. To determine whether this is due to a
greater effect of fluid deprivation on F344 males, 40 F344
and 40 SD male rats were either fluid deprived (1 hr/day
access to fluid) or nondeprived (24 hr access to fluid).
Following the first presentation of a 10% sucrose
solution, a CTA was induced by injection of 0.15 M LiCl
(10 ml/kg). Daily extinction trials began 2 days later
and continued until criterion for extinction (100% of
first day consumption) was reached. The fluid deprived
F344 and SD rats extinguished faster than the nondeprived
rats. However, the percentage increase in the extinction
rates of the deprived F344 was 2-fold greater than that of
the SD. These results suggest that F344 are affected more
strongly by fluid deprivation and have a greater reduction
in sensitivity to T. ONR N0014-J-1296

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APPENDIX J

Chambers, K. C. A neural model for conditioned taste aversions.
Annual Review of Neuroscience, 13: 373-385, 1990.

A NEURAL MODEL FOR CONDITIONED TASTE AVERSIONS

Kathleen C. Chambers

Department of Psychology, University of Southern California,
Los Angeles, California 90089-1061

From the time the parameters defining conditioned food aversions (CFAs, learned aversions to a food or fluid when consumption of that substance is followed by illness) were determined, this learning situation has fallen outside the main conceptualizations of the traditional forms of classical and instrumental learning (Garcia & Koelling 1966, Garcia et al 1966). The two main characteristics that distinguish CFAs from the traditional learning paradigms are learning after a long delay between the food stimulus and the illness (up to several hours) and strong and persistent learning after a single pairing of the food stimulus and illness. It is history, now, that these differences altered the theoretical framework of learning and memory. After the publication and acceptance of the seminal papers of Garcia (Garcia & Koelling 1966, Garcia et al 1955, 1966), there was a flurry of research on CFAs (Riley & Baril 1976). But as is apparently true of all things novel, habituation set in and interest in this area waned. Now with the growth of the field of behavioral neuroscience and the successful application of neurobiological techniques to the study of learning and memory, interest in this maverick of learning is again increasing.

NEURAL MODEL FOR CLASSICAL CONDITIONING: A POOR MODEL FOR CONDITIONED TASTE AVERSIONS

The most significant progress in identifying and characterizing the neuronal substrates of learning and memory has been made for classically conditioned situations, e.g. autonomic conditioning of heart rate (Cohen 1982, Kapp et al 1982) and eye blink conditioning (Thompson 1986). In

these Pavlovian paradigms, a stimulus (unconditioned stimulus, US) that elicits a response (unconditioned response, UR) is paired with another stimulus (conditioned stimulus, CS) that does not elicit the UR. The two stimuli are paired so that they are contiguous and so that the CS can provide information about the US (Rescorla 1988). Learning is inferred when presentation of the CS produces a response similar to the UR. The determination of the neural substrates for this learning situation has involved the identification of four pathways: the US, UR, CS, and CR pathways.

Although CFA learning is thought to be a form of classical conditioning, it does not fit within this four-pathway model. The food stimulus has been identified as the CS, the illness as the US, and the avoidance of the food as the CR. There is, however, no clearly identifiable UR; a CFA can occur without an overt UR (Garcia et al 1972). A three-pathway conceptualization has been implicit in most discussions of the neural basis of CFAs. Discussions have focused on how the food stimulus and illness are integrated neurally to produce a new response to the food. But a detailed analysis of the learning situation for one form of CFA learning, conditioned taste aversion (CTA), suggests that this conceptualization is not adequate.

Taste stimuli have been known to elicit behavioral responses prior to food absorption in addition to the well-known physiological responses, salivation and increased insulin release (Fischer et al 1972, Grill & Berridge 1985, Pavlov 1927, Steiner 1979). The most preferred tastes, such as sweet, evoke increased consummatory responses and the least preferred tastes, such as bitter, evoke reduced consummatory responses and food spillage (Carpenter 1956, Rozin 1967). More recently, Grill & Norgren (1978) have described more complex behavioral responses elicited by different novel taste stimuli in rats. The rats were fitted with an intraoral catheter and the tastes were delivered directly into the mouth. The animals exhibited essentially two different patterns of stereotyped mouth, tongue, head, paw, and forearm movements that reflected hedonic responsiveness to taste. Preferred substances elicited a series of rhythmic mouth movements and alternations between tongue protrusions and tongue retractions that resulted in swallowing and pawlicking (ingestive responses). Nonpreferred substances elicited mouth gaping with tongue retraction followed by long duration tongue protrusion and then tongue retraction and mouth closure. This sequence of responses was repeated several times, resulted in a reduction in swallowing, and was often followed by a sequence of fixed action patterns, which included chin rubbing, head shaking, paw wiping, and forelimb flailing (aversive responses). Other tastes elicited a mixture of these two patterns.

When rats are poisoned after consuming a preferred sweet taste such as sucrose, their subsequent behavioral responses to sucrose resemble those exhibited after consumption of nonpreferred bitter tastes such as quinine. They exhibit decreases in consumption levels, spillage of food and stereotyped aversive responses (Berridge et al 1981, Garcia & Koelling 1966, Rozin 1967). Illness, then, alters the response elicited by taste.

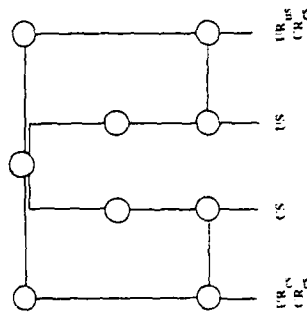
In most classical conditioning situations, the response elicited by the CS is altered because of its association with the US. In some cases the CR resembles the UR quite closely. The CR generally does not, however, become identical to the UR (Holland 1984, Rescorla 1988). The CR can lack the intensity and some of the response repertoire observed for the UR and can include some responses that are not part of the UR. In some cases the CR produced by a given CS is opposite that of the UR, e.g. increases in activity and heart rate elicited by a shock US and decreases in activity and heart rate elicited by a tone CS (de Toledo & Black 1966, Rescorla 1988). Holland (1984) has suggested that the CR is composed of two behavioral elements: one that is similar to or at least in some way appropriate to the US and one that is similar to the response elicited by the CS prior to conditioning (Figure 1).

What distinguishes the CTA learning situation from many other forms of classical conditioning is that the CR is entirely part of the repertoire of elicited responses for the CS sensory modality, in this case, taste. Although the response is appropriate to the US in that illness often produces decreases in food consumption, the decrease in consumption in a CTA situation is not general as in the case of illness, but is specific to the CS. The CR is not similar to the response elicited by the CS prior to conditioning but is opposite that response. Consequently, the function of the US also is different in CTAs than in traditional classical learning. The US does not act as the elicitor of what will become the essential characteristics of the CR. Instead it changes the response elicited by the CS from one form (ingestive) to another (aversive).

PROPOSED NEURAL MODEL FOR ACQUISITION OF CONDITIONED TASTE AVERSIONS

The determination of the neural substrates for CTAs, then, should involve the identification of the following pathways (Figure 1): the US pathway, the CS pathway, the pathway for the elicited response to the CS prior to conditioning (UR_{CS}), and the pathway for the elicited response to the CS after conditioning (CR_{CS}). Each taste is connected to both the ingestive and aversive responses. These connections are probably innate, since

TRADITIONAL CLASSICAL CONDITIONING



CONDITIONED TASTE AVERSIONS

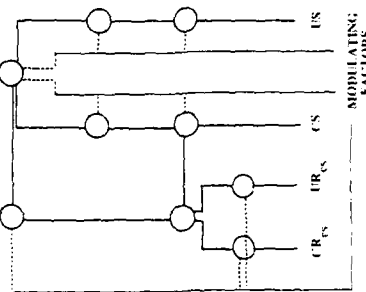


Figure 1. Simplified schematic of a neural model for traditional conditioning and conditioned taste aversion. Abbreviations: CR_{CS} , conditioned response to the CS; CS , conditioned stimulus; UR_{CS} , unconditioned response to the CS; UR_{US} , unconditioned response to the unconditioned stimulus; US , unconditioned stimulus.

hedonic reactions to taste have been observed in prematurely born and full-term neonatal individuals (Steiner 1973, 1979). The relative strengths of the two innate connections are dependent on the given taste. In the case of sucrose, the innate connection to the ingestive response is stronger than the innate connection to the aversive response.

If exposure to a taste is followed by illness, the connection to the ingestive response system will weaken and the connection to the aversive response system will strengthen. It is most likely that the illness-induced changes involve two processes rather than one. Grill & Berridge (1985) have suggested that palatability processing involves two mechanisms and

have provided evidence that the ingestive and aversive response systems can change independently. Thus, in order for the aversive response system to be expressed solely, a weakening of the ingestive response system would have to occur. If exposure to a taste is not followed by negative consequences, a stronger connection to the ingestive response system will result. A stronger connection to the ingestive response system also will occur if a given taste is associated with positive reinforcement or if it is followed by recuperation from illness (Garcia et al 1977, Revusky 1967, 1974, Rozin 1969). So, experiential factors can alter the strengths of the innate connections to the ingestive and aversive response patterns. Thus, after a given taste is experienced, the relative strengths of the ingestive and aversive response systems are a function of the original innate connections, the number of exposures to the taste with illness, and the number of exposures to the taste without illness. This hypothesis is supported by the findings that CTAs to nonpreferred tastes are stronger than to preferred tastes (Fascorn 1973), that repeated pairings of a taste with illness strengthens an aversion, and that repeated pairings of a taste without illness reduces the strength of an aversion (Kalat & Rozin 1973).

Other factors associated with the CS and US can influence the strength of an aversion and therefore must be taken into account when developing a neural model for CTAs. The strength of an aversion has been found to be a function of the intensity of the taste as measured by concentration (Dragoin 1971) and the amount consumed on the first exposure (Bond & Di Giusto 1975), the intensity of the US (Revusky 1968), and prior experience with the US (Cannon et al 1975).

Several factors that are not essential or critical for aversion learning can modulate the development and strength of CTAs. The development and strength of an aversion are dependent on the hormonal milieu and deprivation state of the animal. The presence of testosterone (T) increases the proportion of animals that develop a CTA (Chambers et al 1981), and the presence of dexamethasone attenuates the strength of an aversion (Hennessy et al 1976). Water deprivation reduces the proportion of male rats that develop an aversion (Chambers et al 1981). It is interesting that deprivation can alter the hedonic value of tastes. Foods are reported to be highly palatable with deprivation and unpleasant with satiety (Cabanac 1971). Also, the number of ingestive responses decreases and the number of aversive responses increases as meal termination approaches (Grill & Berridge 1985). So, the relative strengths of the ingestive and aversive response systems are also a function of modulating factors. A complete understanding of the neural mechanisms controlling CTAs would have to include a determination of the neural circuitry for the modulating factors (Figure 1).

KNOWN NEURAL CIRCUITRY FOR CONDITIONED TASTE AVERSIONS

The US Pathway

A number of reviews have examined the US pathways (Ashe & Nachman 1980, Borison & Wang 1953, Coil & Garcia 1977, Kiefer 1985). The vagus nerve conveys information from the gastric-intestinal mucosa primarily to the caudal region of the nucleus of the solitary tract (NST; Torvik 1956). It is then conveyed to the pontine parabrachial nucleus (PBN; Norgren 1978) and the insular cortex (Cechetto & Saper 1987). The area postrema, an area of the brain on the floor of the fourth ventricle that lacks a blood-brain barrier, detects chemicals in the blood. As there are reciprocal neural connections between the area postrema and the NST (Morest 1960, 1967), information about these blood-borne chemicals is probably conveyed to the NST.

A wide variety of substances can be used as the US. The route by which information about these substances is conveyed to the brain varies with the particular chemical and the route of administration. LiCl, a widely used illness-inducing agent, appears to act primarily by way of the area postrema. Lesions of the dorsolateral region of this area attenuate or abolish the learning of taste aversions induced by LiCl (Ritter et al 1980). Vagotomized rats, however, develop essentially normal taste aversions (Martin et al 1978). The vagus nerve mediates copper sulfate induced aversions when this substance is administered intraperitoneally or intragastrically, but when it is given intravenously the area postrema mediates the aversion (Coil & Norgren 1981, Coil et al 1978).

Although the vagus nerve and the area postrema are important routes for many different chemicals, they may not be the only means by which information is conveyed to the brain. The area postrema is an important structure for the induction of emesis when apomorphine is administered, but neither lesions of this area nor vagotomy has an effect on the ability of an animal to learn CTAs (Kiefer et al 1981, Van der Kooy et al 1983), it is not known what effect disruption of both systems has on CTA learning.

The CS Pathway

The CS for CTA learning involves stimuli that are normally used by a given species for the identification of food. For many species taste is the primary stimulus for identification. But it must be noted that other stimuli such as odor can serve as weak cues (Kiefer 1985) and some species use other senses, such as vision in birds, as the primary stimulus (Gaston 1977).

The gustatory pathway has been reviewed recently by Norgren (1984) and Travers & Norgren (1987). In summary, taste receptor cells are located primarily in the tongue and hard and soft palates. Taste information is transmitted primarily to three peripheral taste nerves: the chorda tympani branch of the facial nerve, the lingual branch of the glossopharyngeal nerve, and the greater superficial petrosal branch of the facial nerve. Gustatory afferent fibers from the facial and glossopharyngeal nerves terminate in the ipsilateral NST. Ascending axons from the NST terminate in the ipsilateral PBN in rodents and lagomorphs and in the ventroposteromedial nucleus of the thalamus in primates. The PBN sends projections ipsilaterally to the parvocellular division of the ventroposteromedial nucleus (VPMpc) and also projects extensively to the ventral forebrain, in particular, the lateral hypothalamus, central nucleus of the amygdala, and bed nucleus of the stria terminalis. The VPMpc projects to the insular cortex, which projects back to the VPMpc, central amygdala, PBN, and NST.

The UR₁ and CR₁ Pathways

Neural areas rostral to the PBN are not critical for hedonic reactions to taste. Hedonic responsiveness remains in rats with supracollicular decerebrate preparations that leave only the first (NST) and second (PBN) central gustatory relay nuclei. Intraoral taste stimulation of these rats elicits the same ingestive and aversive patterns of taste responsiveness at the same concentrations as it does in intact rats (Griff & Berridge 1985).

Some neurons in the PBN project to oro-motor nuclei (Travers & Norgren 1983) and respond to both the hedonic dimensions of taste and oro-lingual movement (Schwartzbaum 1983). It seems likely that the ingestive and aversive behaviors are organized entirely in the brain stem and that the control of these behaviors involves the NST and PBN and their polysynaptic connections to the motor neuron pools controlling the behaviors. As the ingestive and aversive movements to taste stimuli are stereotyped, repetitive, and rhythmic, the neural circuits for these behaviors may function as pattern generators, with higher brain systems acting only as modulators. In this sense, the control of the behaviors may resemble that of vertebrate locomotion (Griffner 1985).

Since the behavioral response elicited by bitter tastes is similar to that elicited by a taste that has been paired with illness, one would expect that at least at the level of the behavioral pattern generators, the neural code for the CS would be similar to that for quinine. Chang & Scott (1984) have found that the pattern of activity of sucrose-best NST neurons in response to a sweet taste changes after rats acquire an aversion to this taste so that the activity pattern more closely resembles that of bitter tastes.

CS-US Integration

Possible sites of taste-illness integration have been discussed in a number of recent reviews (Ashe & Nachman 1980, Gaston 1978, Grill 1985, Kiefer 1985). The search for such sites has been plagued by inconsistent findings, and unequivocal candidates have not emerged. The neural control of this primitive form of learning is clearly complex, and there are likely a number of neural routes that can be used for laying down the trace. Despite the contradictory endeavors, some findings have come out of the search that should provide more insight into the neural organization of CTAs.

One of the more consistent findings has been that lesions of the basolateral amygdala disrupt taste aversion learning and retention of an aversion learned prior to lesioning (Simbayi et al 1986, Nachman & Ashe 1974). After finding that cutting the connections between the amygdala and the temporal cortex produced the same deficits as lesions of the basolateral amygdala, however, Fitzgerald & Burton (1983) suggested that it is the destruction of the fibers of passage that produces the deficits after lesions of the basolateral amygdala, not the destruction of the nucleus itself. Recently, Dunn & Everitt (1988) found that ibotenic induced lesions that spare the fibers of passage had no effect on aversion learning. Anna Brownson, Richard Thompson, and I have confirmed and extended this finding in a preliminary study. Electrolytic lesions attenuated both the acquisition of an aversion and the retention of an aversion induced prior to lesioning; neurotoxic lesions (NMDA-induced), which also spare the fibers of passage, had no effect on either acquisition or retention (Figure 2). Clearly the issue of axons of passage is critical to an understanding of neural mechanisms.

Lesions of the PBN disrupt acquisition of a CTA when there is a delay between the CS and US (Schulkin et al 1986, Di Lorenzo 1988), but if there is no delay, animals can learn an aversion (Di Lorenzo 1988). Similar results have been found for lesions of the gustatory cortex (Lorden 1976). These findings suggest that there are different neural mechanisms for learning when CS-US intervals are short and long. Any neural model must include both pathways.

BEYOND ACQUISITION OF CONDITIONED TASTE AVERSIONS

Although I have focused only on the acquisition process, a complete neural model of CTA should include retention or memory storage processes and extinction processes as well. There probably are neural areas that are part of the pathways for all three processes, but the pathways are different.

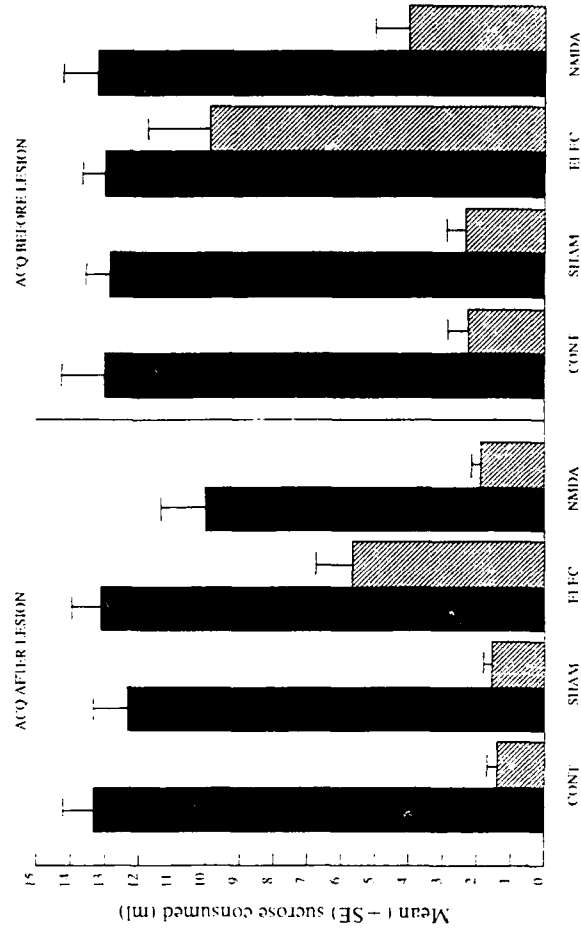


Figure 2. Mean (\pm SE) sucrose consumed by control (CON), sham, electrolytic lesioned (ELFC), and neurotoxic lesioned (NMDA) male rats on the day of acquisition (ACQ; dark bars) and the first day after acquisition (thatched bars) when acquisition was given after and before lesions of the basolateral amygdala.

Each process has its own set of modulating factors that influence that process independently of the other.

Retention

Little is known about the neural mechanisms for CTA, but the data so far suggest that the neural mechanisms for acquisition and retention differ. Although the gustatory neocortex is involved in acquisition, it is not essential (Braun et al 1972, Lasiter & Glanzman 1982, Lorden 1976). It is, however, critically involved in retention of aversions, as animals with these lesions do not retain a previously learned aversion (Braun et al 1981, Yamamoto et al 1981).

Extinction

Extinction has been regarded merely as a reflection of the acquisition process. Indeed, in much of the early Pavlovian and Skinnerian literature, the strength of acquisition was indexed by the rate of extinction. If extinction was slow, acquisition was said to be strong, and if extinction was fast, acquisition was asserted to be weak. Extinction, however, at least of a

CTA, is far more complex than this. Some evidence suggests that the neural processes mediating acquisition and extinction of a CTA are different. If animals are anesthetized or under cortical spreading depression when they are given exposure to a taste, they do not acquire a CTA but they do extinguish a previously learned aversion Burēs & Buresová 1979). The vagus nerve plays a role in extinction that is independent of its involvement during acquisition (Coil et al 1978, Kiefer et al 1981). Rats that are vagotomized prior to or after acquisition of a CTA exhibit a faster extinction than nonvagotomized animals, even when a US (apomorphine) that is not vagally mediated is used. A number of other factors modulate extinction independently of acquisition. The rate of extinction is altered when adrenocorticotropin (ACTH) levels are elevated, when T is present, and when animals are under water deprivation (Chambers 1985, Chambers & Sengstake 1979, Kendler et al 1976, Sengstake & Chambers 1979). ACTH and T decrease and water deprivation increases the rate. It is the presence of these factors during extinction that alters the rate of extinction. Their presence or absence during acquisition of the aversion has no effect.

As suggested decades ago by Clark Hull (1943), extinction is a learning process. Simply stated for CTAs, it is unlearning that the taste predicts illness and learning that it predicts safety (Chambers 1985, Kiefer et al 1981). With respect to the neural model for CTAs outlined above, extinction is a process by which connections to the aversive response system are weakened and connections to the ingestive response system are strengthened. Any information on the subsequent consequences of ingesting the CS is processed. If the consequences are neutral, that information serves to alter the relative strengths of the two response systems. Thus, after a CS has been experienced without negative consequences, the relative strengths of the ingestive and aversive response systems are a function of the relative strengths of these systems after the CTA, the number of exposures to the taste without illness, modulating factors, and probably the original innate predisposition.

CONCLUSION

Since its discovery, students of learning have argued whether CTA fits best in a classical or instrumental learning framework. The fit seemed poor in either case. Although most have placed it within classical conditioning, the issue is still unresolved. Taste aversion learning seems to share the following with traditional kinds of Pavlovian conditioning: a neural integration of the CS and US, a change in the meaning of the CS as a result of this integration so that the CS becomes a signal that predicts the occurrence of the US, and an elicitation of a CR by the CS that is an

anticipation of the occurrence of the US. There are characteristics of CTAs, however, that traditional Pavlovian conditioning does not share, i.e. the ease with which the US and CS are integrated, the ability to integrate despite the long delay between the US and CS and despite intervening CSs from the same sensory modality, the context independence of the CS, and the origin, stemming from the CS, of the CR. It is unclear how critical these differences are, but they certainly alter how one would develop a neural model of learning.

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